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International Conference on the Standardisation of Sera and Serological Tests, 2d, Paris, 1922
Reports on serological investigations

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International Conference on the Standardisation of Sera ... 2nd, Paris, 1922

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Health Organisation

REPORTS

ON

SEROLOGICAL INVESTIGATIONS

PRESENTED TO THE

SECOND INTERNATIONAL CONFERENCE ON THE STANDARDISATION OF SERA AND SEROLOGICAL TESTS

HELD AT THE

Pasteur Institute in Paris in November 1922

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APR 11, 1924

Reports by :

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INTRODUCTION

It will be remembered that the Health Committee of the League of Nations decided, at its meeting in Paris in October 1921, to initiate experimental enquiries on the standardisation of sera and the serodiagnosis of syphilis, as, in spite of the general recognition of the necessity for unification of the standards of antitoxic sera, a still greater confusion had arisen during the late war.

The titration of *anti-tetanic serum* used on a very large scale has been carried out by four different methods of unification.

The practice of standardising *diphtheria antitoxin* in accordance with the Ehrlich unit, preserved in his institute in Frankfort-on-Main, almost universally adopted before the war, had been discarded and it was advisable to re-establish an international unit.

The war and its aftermath had emphasised the epidemiological importance of the use of the *anti-dysentery serum*, but a standard of general application had to be determined for this serum.

The research work carried out during recent years has added greatly to the knowledge of the biology of the *meningococci* group, with the result that its *nomenclature* required standardisation, and it was necessary that the measures for testing the potency of the *anti-meningococcic sera* should be universally readjusted.

For the *pneumococci* also, it was considered to be of equal importance to institute research into the different *types* in their world distribution, as well as to examine more closely the methods of titration of *anti-pneumococcus serum*.

Another and a very difficult problem related to the methods of *serological diagnosis of syphilis*. It was thought desirable to make an attempt to find a practical and, if possible, uniform method.

The Health Committee believed that all these enquiries should be entrusted to State laboratories and National Institutes which are constantly engaged in the investigation of such problems, and invited the following State Institutes to take part in the laboratory researches required to establish the standardisation of sera :

	Research work in which these Institutes and Laboratories already specialise
GREAT BRITAIN.	
Medical Research Council.	Anti-dysentery serum. Anti-meningococcus serum. Serodiagnosis of syphilis.

	Research work in which these Institutes and Laboratories already specialise
FRANCE.	
Pasteur Institute, Paris.	Anti-tetanic serum. Anti-diphtheritic serum. Anti-meningococcus serum. Anti-pneumococcus serum. Anti-dysentery serum.
ITALY.	
State Institute, Rome.	Anti-diphtheritic serum. Anti-tetanic serum.
POLAND.	
State Epidemiological Institute, Warsaw.	Anti-dysentery serum. Serodiagnosis of syphilis.
SWITZERLAND.	
Hygienic Institute, Basle.	Anti-dysentery serum.
BELGIUM.	
Pasteur Institute, Brussels.	Serodiagnosis of syphilis.
JAPAN.	
Kitasato Institute, Tokio.	Anti-dysentery serum.
UNITED STATES.	
Hygienic Laboratory, Washington.	Anti-tetanic serum. Anti-diphtheritic serum.
Rockefeller Institute.	Anti-meningococcus serum. Anti-pneumococcus serum.
AUSTRIA.	
Serological Laboratory, Vienna.	Serodiagnosis of syphilis.
GERMANY.	
Institute of Experimental Pathology, Frankfort.	Anti-diphtheritic serum. Anti-tetanic serum. Serodiagnosis of syphilis.
Cancer Research Institute, Heidelberg.	Serodiagnosis of syphilis.
Robert Koch Institute, Berlin.	Anti-meningococcus serum. Anti-pneumococcus serum.

THE FIRST INTERNATIONAL CONFERENCE ON THE STANDARDISATION OF SERA AND SEROLOGICAL TESTS

In order to discuss the programme, it was decided to convene a Conference of scientists who specialise in the different branches This First International Conference on the Standardisation. of Sera and Serological Tests was held, at the invitation of the

Health Committee of the League of Nations, on December 12th to 14th, 1921, at the British Ministry of Health, London.

The Conference decided to constitute the following Sub-Committees :

Sub-Committee No. 1	Diphtheria and Tetanus.
Sub-Committee No. 2	Meningococcus and Pneumococcus.
Sub-Committee No. 3	Dysentery.
Sub-Committee No. 4	Serodiagnosis of Syphilis.

Anti-diphtheritic Serum.

The First International Serological Conference held in London in 1921 adopted the following resolution concerning diphtheria :

Two units are used at present.

Between these two units, namely, the unit of Ehrlich preserved at the Frankfort Institute and the American unit as supplied by the Washington Bureau, according to the experiments carried out to date, there are only very small differences. To determine these differences accurately, the standard sera and test toxins for the necessary researches will be given to the various participating institutes both by the Frankfort Institute for Experimental Pathology and the Washington Public Health Bureau.

The result of the experiments performed with these sera and toxins at the several laboratories represented will be delivered to the Danish State Serum Institute, which for this purpose will act as the central laboratory of the Health Committee of the League of Nations.

Experiments were started immediately by the Institute for Experimental Pathology, Frankfort, the Pasteur Institute of Paris, the State Bacteriological Laboratory of Rome, the Hygienic Laboratory, Washington, and the Danish State Serum Institute. The above Institutes had compared the Ehrlich unit which is kept by the Frankfort Institute and the antitoxic unit which is kept by the Washington Bureau, and they demonstrated by preliminary experiments that the difference between these two units was almost inappreciable.

Meeting of the Sub-Committee on Diphtheria and Tetanus held in Geneva in September 1922.

By midsummer 1922 sufficient progress was realised to justify the Sub-Committee on the anti-diphtheritic and anti-tetanic sera being convened at Geneva, from September 25th to September 27th, in order to compare the results obtained from the study of these sera. The various institutes that had undertaken comparative research, on the invitation of the Health Committee, were represented by the following persons :

Chairman of the Health Committee : Dr. Th. Madsen, Director of the Serotherapeutic State Institute, Copenhagen.

Chairman of the Sub-Committee on Anti-diphtheritic and Anti-tetanic Sera : Professor Louis Martin, Deputy Director of the Pasteur Institute, Paris.

Professor B. Gosio, Director of the Bacteriological Laboratory, Public Health Service, Rome.

Professor W. Kolle, Director of the Institute of Experimental Pathology, Frankfort.

Dr. G. W. McCoy, United States Public Health Service, Director of the Hygienic Laboratory, Washington.

Professor M. Miyajima, of the Kitasato Institute, Tokio, and Professor Tarassevitch, representing the State Institute for the Control of Sera, at Moscow, also attended the Conference.

The Conference was unanimous in the belief that it was evident, from the reports on the results obtained, that the Ehrlich method for the titration of diphteria anti-toxin should be finally adopted and that the anti-toxic units at present prepared at Frankfort and at Washington could be used as the standard for the measurement of the *diphtheria* anti-toxin.

The Conference therefore resolved that :

In order to ensure that the sera used as standards by the various States have identical titre, it would be well if Professor Madsen, acting as Director of the Serotherapeutic State Institute of Denmark, which Institute has been designed to act for this purpose as a central laboratory for the Health Committee of the League of Nations, should arrange for comparative studies of such sera to be carried out every year.

In order to carry out the results of the Geneva Conference, the Danish State Serum Institute will receive periodically specimens of the standard antitoxin for the comparative tests. It is obvious that any State Institute on application to Copenhagen will be able to receive an authentic sample of the standard.

The Danish State Serum Institute will examine the sera used as standards by the various States at least once a year. Should any variation be detected, it will be suggested to the Institute concerned that comparative tests be carried out. It is further understood that any Institute will be free to send to Copenhagen samples of its standard serum at any time with the request that they should be tested against the international standard.

The Second International Conference on the Standardisation of Sera and Serological Tests.

The Chairman of the Health Committee, considering from the results of the reports on all subjects under investigation, sent him by the various institutes and laboratories, that the progress of the researches called for a meeting of representatives of the participating laboratories and institutes, in order to compare the results obtained, and to settle the details of the further researches to be carried out, convened for November 1922 a Second International Conference on the Standardisation of Sera and Serological Tests.

The Conference was held from November 20th to 26th, 1922, at the Pasteur Institute in Paris.

The Conference adopted the following resolution concerning diphtheria, which has been drawn up by the Sub-Committee on Anti-diphtheritic and Anti-tetanic Sera.

Anti-diphtheritic Serum.

The Sub-Committee adopted the proposals contained in Dr. Madsen's report on the Geneva Conference.

The Sub-Committee decided that, as the Ehrlich method for the measurement of the international unit, has been adopted, it would be very desirable to induce firms in all countries to supply phials containing a fixed quantity of anti-toxic units for preventive and therapeutic injections.

The Sub-Committee resolved that, in the first place, an enquiry should be made with a view to ascertaining what quantities of antitoxic units are at present contained in the phials offered for sale.

It was decided that the Health Committee of the League of Nations should ask the various institutes and firms for samples of phials and have a report on the enquiry prepared for a later session.

Anti-tetanic Serum.

The First International Serological Conference held in London in 1921 adopted the following resolution concerning tetanus :

Four methods are employed at present to describe the potency of anti-tetanic toxin.

These four methods start from different points of departure and experiments have not yet determined the exact relation between the units determined by each of these methods.

The Sub-Committee considers, however, that it is desirable and possible to establish a common measure by an agreement on a single standard antitoxin, using the principle which has been adopted in connection with the standardisation of anti-diphtheritic serum.

In the first instance, the participating laboratories must fix experimentally the relations which obtain between the four units at present employed.

With this object, an exchange of sera and toxins will be made in order to secure the necessary comparative experiments.

The details of the experiments made with each method will in all cases be described in precise detail.

All the relative documents will be sent to the Danish State Serum Institute, which for this purpose will act as the central laboratory of the Health Committee of the League of Nations, and the results will be discussed at a subsequent conference.

Comparative experiments have been carried out by the Institutes at Copenhagen, Frankfort, Paris, Rome and Washington.

Although the experiments have not been carried to the final stage, sufficient progress has been made to enable an understanding to be reached as regards the basis of an international agreement.

The Sub-Committee on Anti-tetanic Serum met in Geneva on September 25-27, 1922.

A comparison was made of the results of the tests, which showed that the ratio between the different sera was approximately as follows :

Taking the German serum as being 1 titre, the American would be 60 to 66 titre and the French in the neighbourhood of 2,500 titre.

All the members of the Geneva Conference decided that a unit should be adopted which would be intermediate of the two extremes.

Had the ratio been exactly 1 : 50; 2,500, it would have been possible to accept it at once as the most convenient common measurement. As, however, this is not the case, it was obvious to the members of the Geneva Conference that, in order to attain this simple proportion, the units used at present would have to bee re-adapted.

In order to attain this, Professor Kolle has offered to propose to his Government that the German unit should be so adapted that its relation to the American unit should be exactly 1 : 50. Moreover, he declared his intention of proposing that this newly established unit should be multiplied by 100 in order to make it conform to the measurement of the diphtheria antitoxin, so as to meet the convenience of the medical practitioners.

If this be accepted, then the following ratio would obtain :

American unit.	New German unit.	French unit.
50	100	2,500

Prof. Louis Martin observed that, under these conditions, the ratio between the American and the new German unit would be 1 to 2 and he therefore moved that the American unit multiplied by two, or, in other words, the new German unit, be accepted as the international standard.

The Sub-Committee proposed that the anti-tetanic sera be titrated against the standard established in accordance with the accepted principles as set out above.

Thus the Conference has adopted in principle an international unit. There remained only the necessity of establishing definitely by a series of tests the exact relationship between the several sera used for the measurement of the antitoxic content of the anti-tetanic serum.

The Conference, moreover, asked that only procedure based on the neutralisation of toxin by tetanic anti-toxin should be accepted.

The Danish State Serotherapeutic Institute will continue to act as central laboratory for the Health Committee of the League of Nations for the research necessitated by this proposal.

The decision of the Geneva Conference implied the necessity of further experiments in order to define in detail the technique to be used for the titration of anti-tetanic serum with the new

standard. Moreover, the preliminary experiments which have been carried out hitherto have brought to light certain experimental facts which will be investigated further by the Institutes concerned before definite results can be obtained.

Anti-meningococcus Serum.

The First International Serological Conference held in London in 1921 adopted the following resolution concerning antimeningococcus sera :

1. With the object of maintaining full information regarding the various types of meningococci, the several laboratories mutually agreed to exchange agglutinating sera and strains of meningococci, the latter being obtained exclusively from the cerebro-spinal fluid of meningitis cases.

2. Macroscopic methods will be employed in agglutination experiments, the bacterial emulsions having been kept for 24 hours at a temperature of 37° C., without prejudice to other methods which may also be employed. Investigations into the saturation of agglutinins may be carried out by any method.

3. In the existing state of our knowledge, and without prejudice to the opinions of the several Institutes participating in these researches and who are not represented at this Conference, it appears to be difficult to determine the therapeutic value of antimeningococcus serum by measurement of agglutinins, sensitizers (*sensibilisatrices*) and opsonins.

4. Fresh experiments will be undertaken in the various laboratories regarding the value to be attached to the determinations of antiendotoxic and bactericidal power.

Six Institutes participated in the research — the Laboratory of the Ministry of Health, London, the Medical Research Council, the Pasteur Institute of Paris, the Danish State Institute, the Rockefeller Institute, and the Robert Koch Institute of Berlin; but, owing to the low prevalence of epidemic cerebro-spinal meningitis during the last year, these researches could not be carried out on a large scale.

The study on the *Meningococcus types* in Denmark led to the conclusion : (1) that in nearly all cases the type A of the Danish classification was the pathogenic type in Denmark; (2) that the Danish type A corresponds to Gordon type II (although it is not quite identical).

The comparison of the types A, B, C, D of the Pasteur Institute with the Gordon types carried out in the Pasteur Institute of Paris showed that the Gordon types 1 and 3 correspond to the type A of the Pasteur Institute, and Gordon types 2 and 4 to type B of the Pasteur Institute.

The Pasteur Institute's types C and D proved to be specifically different from the Gordon's types.

So far it was not possible to collect a sufficient number of strains for investigation regarding the occurrence of different types of meningococcus in Germany.

It appeared from the researches undertaken on the *meningococcus serum* that none of the methods which are in use at present was likely to be accepted by all the workers as sufficiently reliable to serve as a definite measure of antibody content or of curative value. In Professor Madsen's view the complement fixation test, as a common denominator for the different antibodies, would be the method most likely to express its strength. Professor Dopter obtained constant results by the so-called peritoneal test in the determination of the bactericidal power. Dr. Wadsworth pointed out that the agglutinative titre unit is the official standard of the New York State.

The problems requiring further study as regards the uniform method of international classification of the type of meningococci and of methods for testing the potency of Anti-meningococci serum are embodied in the following resolutions drawn up by the Sub-Committee for Anti-meningococcus serum and adopted by the Paris Conference.

In order to secure a more uniform international classification and nomenclature for meningococci, the Sub-Committee resolves :

I. That an interchange of meningococcus cultures and monovalent anti-meningococcus sera be made between the different laboratories. Cultures representative of the different types will be selected by the different laboratories, and monovalent serum prepared in animals from each of these cultures.

II. That the classification of meningococci should be based as far as possible :

(1) Upon a primary division into groups to be designated by numerals, for example : I, II (according to the present type classification into types of Gordon), without prejudice to other groups which may be recognised later;

(2) Upon a subdivision of the main groups into sub-groups or types to be designated by letters, together with the numeral of the main group under which they are to be classified, for example Ia, IIb, etc.

The primary division into groups is to be based upon agglutination alone. The groups comprise cultures which are agglutinated similarly in high dilution of monovalent sera which are relatively free from heterologous agglutination.

The secondary division into types is to be based upon distinctive characters as determined by the results of both the agglutination and absorption tests with monovalent serum.

Cultures which cannot be accurately identified should be classed under group "X".

For the study of the standardisation of therapeutic sera, the Sub-Committee further resolves :

(1) That a new series of tests shall be made with monovalent and polyvalent sera, to determine their comparative content in agglutinins, amboceptors, opsonins and antitoxin. The serum used should be collected at different intervals after the last injection. The serum must be obtained from the horse and must not be heated.

Precise information must be given regarding the method of

preparation, the date of bleeding, the addition of antiseptics and, if possible, the therapeutic action of the same serum in human cases of meningitis.

So far as possible, the same culture should be used for immunising the horses and for testing the serum.

Only meningococci obtained from the cerebro-spinal fluid of cases of meningitis shall be used for this study.

(2) That studies should be made of the effect of heat, especially tyndalisation (56 degrees Centigrade for one hour during three successive days), on the properties of the serum.

(3) That careful studies should be made of the immunological reactions with meningococci recently isolated from cases of meningitis in which the serum has not been effective.

(4) That exchange of cultures and sera should be made through the medium of the Central Institute at Copenhagen.

Anti-pneumococcus Serum.

The First International Serological Conference held in London in 1921 adopted the following resolution concerning anti-pneumococcus serum :

1. A mutual exchange of cultures of different strains of pneumococci will be effected in the same manner as agreed upon in the case of meningococci.

2. Researches into the agglutinating power of anti-pneumococcus serum is of no value in determining its therapeutic power.

The best method of titrating the serum is the measurement of the bactericidal power in animals, preferably mice.

3. New investigations will be carried out with regard to :

(*a*) The best method of inoculation—peritoneal or subcutaneous—for the titration of serum;

(*b*) The selection of methods, preventive or simultaneous, for the injection of sera and cultures;

(*c*) The monovalence or polyvalence of the different sera.

Six Institutes took part in the research work and presented reports on the results obtained to the Second Conference in Paris : the Laboratory of the Ministry of Health, London, the Medical Research Council, the Pasteur Institute of Paris, the Danish State Institute, the Rockefeller Institute, the Robert Koch Institute of Berlin.

Investigations concerning the occurrence of different *types* of pneumococci have confirmed the three chief types I, II, III, defined by the Rockefeller Institute.

The work of the Danish State Serum Institute demonstrated the same relative grouping of types of pneumococci in Denmark as in the U.S.A. (Madsen).

The results obtained in Great Britain by the Medical Research Council and the Laboratory of the Ministry of Health, London, were also in fairly close agreement with the results obtained in the U.S.A. (Griffith and Glynn).

As regards the technique for the determination of type the same results were obtained with the agglutination test and with the complement fixation test after absorption (Madsen).

The Sub-Committee decided that further investigations were necessary with regard to :

1. The distribution of types of pneumococci.

2. The therapeutic action of sera in the treatment of early cases of pneumonia.

3. The mechanism of recovery and the mode of action of the antipneumococcus serum.

4. The antistreptococcus serum.

5. The interchangeability of types and serological modification of pneumococci.

The following resolution was unanimously adopted :

I. The Sub-Committee regards the classification of pneumococci into different serological types as fundamental for all further researches on serotherapy, vaccination and epidemiology, and accepts the three chief types I, II and III, defined by the workers of the Rockefeller Institute, as standard types for international research. Strains not conforming to any of the above three types should be classified provisionally in a group "X".

As pneumococci in certain circumstances may undergo changes in their immunological characters, the classification of the types for epidemiological purposes should be based upon the agglutination reactions with freshly isolated strains, or preferably upon the results of the test with the peritoneal exudate of a mouse injected with sputum or other material taken directly from the patient.

II. With regard to the distribution of the types of pneumococci in different parts of the world, the Sub-Committee expresses the wish that, in addition to further researches in Europe and the United States, investigations along the same lines should be undertaken in tropical and other countries where the climatic and social conditions differ widely.

In the first place, cases of lobar pneumonia should be studied, but observations on other forms of pneumococcal infection should also be recorded.

III. A study of the therapeutic action in the treatment of early cases of pneumonia should be made with anti-pneumococcus serum which has been carefully titrated and found to be highly protective for mice. The investigation should be made only in institutions fully equipped for such a purpose.

IV. Without expressing an opinion in regard to the therapeutic value of the serum in the treatment of pneumonia, the Sub-Committee regards the titration test in the mouse, when controlled by a standard serum, as a basis for an international standardisation of the serum.

Therapeutic serum may be titrated in mice either by injecting intraperitoneally a fixed quantity (0.2 c. c.) of serum, mixed with a highly virulent broth culture in doses ranging from 0.1 c. c. to 0.4 c. c. or more; or by injecting smaller and varying quantities (0.001 or 0.0001 c. c.) of serum intraperitoneally, and three hours later a moderate dose of culture, for example, 0.0001 c. c. The virulence of the culture should

be such as to kill mice in doses of at least 0.000.0001 c. c. within forty-eight hours. For every titration a standard serum must be used.

V. Further research should be made into the mechanism of recovery from pneumonia and into the mode of action of anti-pneumococcus serum.

VI. A study of anti-streptococcus serum is desirable.

VII. Standard cultures, agglutinating and standard therapeutic sera, to be supplied by the Central Institute of Copenhagen.

Dysentery

The First International Serological Conference, held in London in 1921, adopted the following resolution concerning dysentery :

1. It was agreed that it would be advisable that the different institutes, laboratories, etc., should exchange samples of serum and toxin and that further experiments should be carried out, using for these :

(*a*) Different methods of titration;

(*b*) Different species of animals.

2. In testing the potency of sera, there was a general agreement that the antitoxin, especially the antiendotoxin, should be estimated, and there was general agreement that this could be accurately carried out equally well with dead bacilli as with toxin.

In reference to the method of testing the dysentery serum on mice, it was recommended that a series of tests should be carried out on mice, rabbits and guinea-pigs with the standard serum which the Frankfort Institute would supply, comparing them with methods in use in the various Institutes, and that a report on these experiments should be presented at the next Conference. But it was considered that it was advisable also to test the antimicrobic power of serum, employing living bacilli for the tests.

The research work was carried out by the Medical Research Council, the Pasteur Institute of Paris, the Hygienic Institute of Basle, the State Epidemiological Institute of Warsaw and its branch at Cracow, and by the Kitasato Institute of Tokio. Professor Tarassevitch, of the State Serum Control Institute at Moscow, Professor Cantacuzène (Chairman), Professors Kolle and Sachs attended the meetings of the Sub-Committee.

The results obtained were discussed by the Paris Conference, and the following plan of further work has been drawn up by the Sub-Committee :

(1) It is necessary to test further and to determine the choice of the animal to be used for standardisation of anti-dysentery serum.

These tests should be carried out comparatively on rabbits of 1,500 to 1,600 grammes weight and on white mice of 15 to 16 grammes weight.

(2) The substances compared will be liquid toxin, solid toxin (Wellcome) and microbes killed by heat.

(*a*) Liquid toxin will be prepared by each laboratory with a culture of toxic Shiga which will be supplied by the Pasteur Institute (the filtrate of a twenty-days' old culture on peptone broth [Chapotaut's

peptone] sown in litre flasks containing 500 c. c. of liquid with an alcalinity Ph 7.4).

(*b*) The solid English toxin will be supplied by the Wellcome Laboratories.

(*c*) The microbes killed by heating at 56° C for one hour and dried in vacuum will be supplied by the Pasteur Institute (agar culture emulsified in physiological salt solution).

The effect of subcutaneous, intraperitoneal and intravenous injections will be compared.

(3) There is no need, in the tests, to discriminate between the anti-neurotoxic and the antienterotoxic power of the serum.

(4) The titration of sera will be carried out by a mixture of toxin and serum, after 30 minutes' contact at laboratory temperature.

(5) There is no need to try to give the serum an anti-infectious power as distinct from its antitoxic power.

(6) It would be of interest to test on man the power of monovalent (anti-Flexner, anti-His, etc.) sera against homologous strains of dysenteric organisms.

The Institutes now participating are as follow :

Medical Research Council, LONDON.
Ehrlich Institute, FRANKFORT.
Pasteur Institute, PARIS.
Epidemiological Institute, WARSAW.
Institute of Hygiene, BASLE.
Institute of Public Health, Moscow.
Institute of Experimental Medicine, BUCHAREST.
Kitasato Institute, TOKIO.
State Serum Institute, COPENHAGEN.

SERO-DIAGNOSIS OF SYPHILIS.

The First International Serological Conference held in London in 1921 adopted the following resolution concerning the serodiagnosis of syphilis :

1. In a certain number of Institutes, the Wassermann reaction as practised in them should be compared with the methods of Sachs-Georgi, Meinicke No. 3 and Dreyer ("Sigma").

2. The number of cases examined in each Institute should be 1,000 of undoubted syphilis and 1,000 in winch syphilis could be excluded as far as possible.

3. It is also recommended that the different methods should be compared on about (one and the same) 50 cases out of the 1,000, at different stages of the disease. These repetition tests should be carried out on at least three or four occasions in the course of the disease, and it is recommended that the fifty cases should comprise, especially, suspected syphilis of the nervous system and of the eye.

4. The worker should be allowed the opportunity of studying the respective authors' own methods in the respective institutes.

5. The places where the tests are to be carried out should be offi-

ciently equipped and the serologist should be in as close touch with the clinician as possible.

6. For the flocculation tests, the serologist should use only those extracts prepared or controlled by their respective authors.

7. The samples of serum to be tested should be known only by numbers, and the serologist should not have access to the clinical or pathological records until the whole series of tests is concluded, but the chief of the laboratory may cause any serum to be re-tested without making the serologist aware of the fact.

8. A preliminary report should be submitted after the first 500 tests have been concluded.

9. All reports on tests should include information as to :

(*a*) Reliability of the method;
(*b*) Complexity of technique;
(*c*) Relative consumption of time by the method;
(*d*) Expense;
(*e*) Ease and accuracy with which the reaction can be observed;
(*f*) Percentage of dubious results;
(*g*) The extent to which the method yields quantitative results.

10. It is recommended that all the results shall be finally submitted to the State Serum Institute, Copenhagen.

Ten reports were presented : by Prof. MADSEN, KOLLE' Dr. RENAUX, Prof. HIRSZFELD, Dr. GASIOROWSKI, Prof. MÜLLER, Prof. SACHS, Prof. DREYER, Dr. HARRISON and Dr. WYLER. The reports emanated from the following institutes :

Medical Research Council; Danish State Serum Institute; Pasteur Institute, Brussels; State Epidémiological Institute at Warsaw and its branch at Lwow; Institute of Experimental Pathology, Frankfort; Cancer Research Institute, Heidelberg; Pathological Institute, Oxford; Serological Laboratory of the University at Vienna.

I. The following table shows the percentage of positive reactions obtained through the Wassermann test and the flocculation methods of Sachs-Georgi, Meinicke and Dreyer-Ward by the different workers in syphilitic subjects :

Methods	Madsen	Kolle	Renaux		Hirszfeld	Müller	Sachs	Dreyer Ward Mcfutosh Fildes	Harrison Wyler
B.-W. .	59.3	37.2	45	55	47.7	69	54.4	68.9	50.0
S.-G. .	53	33.4	44	54	46.8	46	51.7	—	65.2
M. . .	42.1	33.6	38	40	44.5	54	43.8	—	58.8
Σ . . .	77.7	—	46	74	44.2	61	51.4	79.1	63.1

This table shows that the results of the different reporters are discordant. The highest percentage given by the Sigma test were found by Drs. MADSEN, RENAUX, DREYER, HARRISON and WYLER. On the other hand, the B. W. test has given the greatest

proportion of positive reactions with Drs. Kolle, Hirszfeld, Müller and Sachs. The percentage of very feeble positives obtained by Prof. Kolle is explained by the fact that this authority has chosen cases of primary and of latent syphilis for his tests.

II. The following table shows the percentages of positive reactions obtained with the sera of persons presenting no signs or history of syphilis :

Methods	Madsen	Kolle	Renaux	Hirszfeld	Müller	Sachs	Dreyer	Harrison & Wyler
B. W. .	0	3.8	0	-	1.1	0.5	0.7	0
S.-G. .	0.6	3.9	3	—	1.03	0.16	—	1.8
M. . .	0	3.3	1.5	—	1.7	0.16	—	2.2
Σ . . .	0.3	—	1.9	—	2.75	0.05	0.7	2.7

Some of the reporters give details of cases in which non-specific reactions have been given. Thus Prof. Hirszfeld says that he has found a positive reaction sometimes in malarial and relapsing fevers. On the other hand, the S.-G. reaction has sometimes been positive in cases of tuberculosis.

Dr. Renaux has never seen non-specific reactions with the B. W., but, on the other hand, has seen positive or weakly positive non-specific reactions, 18 times with the S.-G. test, 7 times with the M. and 10 times with the Sigma test. These positive results occurred in 8 tubercular subjects, some hemiplegies, some with dilatation of the aorta, and others. It should be noted that hemiplegia and dilatation of the aorta are often the result of syphilis.

Profs. Sachs and Müller were not prepared to say with confidence that all their control cases giving no specific results were not syphilitic.

Dr. Harrison explained, with regard to the control cases giving positive reactions in his and Dr. Wyler's series, that two which gave positive reactions with all the flocculation tests gave doubtful reactions with the B. W.

One which gave a positive reaction with the Sigma was quite possibly a case of primary syphilis, but had left the clinic and had not yet been traced.

The sera of two cases which gave positive Sigma reactions had been re-tested at later dates, with the results that one gave a clean negative and one gave 1.3 units.

The remaining serum giving a positive Sigma had not been re-tested.

Two of the sera giving a positive S.-G. gave also a doubtful B. W. Of the other two cases, one was re-tested and gave a doubtful S.-G. The remaining serum was from a case of gonococcal arthritis.

III. Certain reporters do not discuss doubtful reactions; others

class them either as weakly positive or else negative. Dr. Hirszfeld, in particular, considers that for statistical purposes doubtful results should be regarded as positive. Dr. Renaux considers that they should be entered as negative in the absence of any history of syphilis.

IV. In the reports presented we find the following replies as to the question : By which method can one obtain a quantitative titre of the positivity of the serum most surely and constantly? —

Dr. Madsen says definitely that the Sigma test gives the most certain and constant results.

Dr. Renaux does not give a definite reply; as regards the S.-G., he thinks further research is necessary; he finds a parallelism between the Sigma and the B. W.

Dr. Gasiorowski considers that the Meinicke test can afford a quantitative result, and Prof. Müller employs three quantities of serum for the M. test. Prof. Sachs and Prof. Müller do not think that the quantitative titration is necessary in any but a comparatively small proportion of cases.

Drs. Harrison and Wyler think that the Sigma test can afford the longest range of titre, but that further research is necessary to determine the relation between the titre and the activity of the syphilitic process.

V. All reporters are agreed that the B. W. test is the most costly in animals, such as guinea-pigs, rabbits and sheep.

VI. Most of the reporters consider the flocculation tests easier to carry out than the B. W.; at the same time, certain of them, notably Drs. Hirszfeld and Renaux, complain that the reading of the results is much more difficult in the flocculation tests and is sometimes even very fatiguing. Dr. Gasiorowski alone finds the Meinicke reaction the easiest of all the reactions to read.

VII. The only reporter who is sufficiently affirmative in his conclusions is Prof. Madsen. While not ignoring the value of the B. W. test, he shows a marked preference for the Sigma test, and suggests that it would be a good plan to make a fresh comparison between these two methods in further research. All the other reporters agree in stating that, in the serodiagnosis of syphilis, the most important factor is the selection of the extract and the method of its dilution.

Prof. Kolle states that discordant results in all serodiagnostic tests depend solely on the antigen; it is essential to study this question more closely, and every serum should be tested by whatever process with at least three extracts.

Dr. Hirszfeld agrees with the other reporters as to the importance of extract and thinks that the question of extract standardisation ought to be considered.

Dr. Renaux expresses the same view and suggests that, in the testing of every serum, the B. W. test should be applied as well as the flocculation.

* * *

After a full discussion, the Second Serological Conference adopted the following resolution concerning further research on the serodiagnosis of syphilis :

(1) The comparison of the four tests shall be continued as follows :

(*a*) The technique of the Bordet-Wassermann test carried out in the laboratories taking part in the research shall be that which has been hitherto employed by them.

(*b*) The Sachs-Georgi test shall be carried out in the same number of tubes as hitherto, but two quantities of sera and one extract will be used, as determined by the special Sub-Committee on Technical Details.

(*c*) For the purposes of this comparison the quantitative titre of the reaction obtained by the Sigma test shall be omitted. Only qualitative results will be considered.

(*d*) The following laboratories shall be invited to make further studies as to the Meinicke test in consultation with Dr. Meinicke :

Heidelberg (Prof. SACHS);
Vienna (Prof. MÜLLER);
Copenhagen (Prof. MADSEN).

(*e*) The laboratories taking part in the whole research shall be invited to study comparatively the influence of the various factors in the tests, particularly variations in the antigen.

(*f*) It is understood that the research will be carried out on 500 cases in which syphilis has as far as possible been excluded and on 500 cases of syphilis. As many as possible of these cases should be in the early primary, in the latent or in the tertiary stages of syphilis.

(2) The State Serum Institute of Copenhagen will endeavour to collect 500 specimens of syphilitic serum and 500 of non-syphilitic. These specimens will each be divided into five equal portions, one of which will be sent to each of the following laboratories :

In Austria, Prof. MÜLLER;
In Belgium, Prof. BORDET;
In Germany, Prof. VON WASSERMANN;
In Great Britain, Dr. HARRISON;
In Poland, Prof. HIRSZFELD,

so as to compare the results obtained by each of these laboratories, employing their normal technique for the Bordet-Wassermann test.

REPORT

OF THE SUB-COMMITTEE ON TECHNICAL DETAILS

I. SACHS-GEORGI TEST.

The serum, after inactivation, is to be diluted to 1 in 5 with 0.85 per cent. salt solution. Of this dilution place :

In No. 1 tube 1.0 c. c.
In No. 2 tube 0.5 c. c.
In No. 3 tube 1.0 c. c.

Add to No. 2 tube 0.5 c. c. physiological salt solution; in No. 3 tube 1.0 c. c.

One extract will be used. This will be supplied by Prof. SACHS.

Dilution of extract. — If the interval of 30 seconds between the addition of the first and second quantities of saline results in flocculation of the diluted extract, it may be shortened sufficiently to prevent this occurrence.

Incubation to be for 20 to 24 hours at 37° C. in the air incubator.

II. SIGMA TEST.

(*a*) Five tubes will be used. In the event of the amount of serum being insufficient, the first tube may be omitted. If then the result is positive, it may be recorded; if it is negative, it must be omitted from the series.

(*b*) *The extract* shall be that supplied by Professor Dreyer and shall be diluted precisely as directed in the description of the method published by Dreyer and Ward.

(*c*) *Incubation.* — During the incubation, it is imperative that the tubes shall be immersed in such a manner that no less than half and no more than three-quarters of the column of fluid in each tube is below the level of water in the water-bath. Incubation is to proceed for at least 20 to 22 hours, and in the case of doubtful reaction to be prolonged to 40 to 44 hours.

SIGMA:

Serum Control. — If the amount of serum permits, a control containing:

Serum, 10 drops;
Alcohol, 3%, 15 drops in saline,

may be used.

If a sufficient amount of flocculation occurs in this control to vitiate the test in the opinion of the serologist, the result will nevertheless be returned, but with a note stating that the control showed flocculation and the amount of flocculation.

Whenever a control is used, the reading of the control tube will be reported.

III. RECORDING OF RESULTS.

Exact directions will be circulated from Oxford and from Heidelberg as to the readings which are to be regarded as positive and negative respectively.

IV

It is desirable to study the influence of the several factors named below :

(1) Comparison between the extracts of Sachs and Dreyer-Ward in the two methods.

(2) Influence of the method of diluting the extracts in the two methods.

(3) Comparison between the use of the thermostat and the water-bath for incubation.

(4) Comparison of the effect of inactivation of the serum for different periods (half-an-hour and one hour and a-half).

(5) Comparison between the use of narrow tubes (Dreyer) and of ordinary tubes (Sachs-Georgi).

(6) Comparison of the results obtained by reading of the tests by the two methods after 20 to 24 hours and 40 to 48 hours respectively.

(7) Study of the relative quantities of serum and extract to be employed.

(8) Comparison of the use of pipettes and drops for measurement.

I — DIPHTHERIA AND TETANUS

DIPHTHERIA ANTITOXIN.

A COMPARISON OF THE GERMAN AND AMERICAN UNITS

By Th. Madsen,

State Serum Institute, Copenhagen.

Based on Experiments by L. E. Walbum.

This comparison was carried out by measuring both standard sera (that of Frankfort and that of Washington) by means of standard toxin, partly prepared in the State Serum Institute at Copenhagen and partly received from Frankfort.

Our intention was to determine the L † of the two toxins by the use of these two sera.

The mixtures used consisted of 1 A. U., and the amount injected never exceeded 4 cc. The mixture was kept at room temperature for one hour and then injected subcutaneously into guinea-pigs of 250 gr. weight. The guinea-pigs were under observation for four days. The results are shown in the following tables :

Danish Test Toxin + Frankfort and Washington Standard Sera.

Frankfort Serum (received March 21st, 1922).

	Toxin cc.	Weight of guinea-pigs gr.	2nd day gr.	3rd day gr.	4th day gr.	L † =
	—	—	—	—	—	—
2/III, 1922	0.80	230	220 inf.	210 inf.	200 diseased	0.82
	0.82	230	210 »	† 2½		
	0.84	230	220 »	† 2½		
	0.86	230	† 1½			
6/III, 1922	0.78	240	220 inf.	210 inf.	200 inf.	0.80
	0.80	255	255 »	240 »	210 † 4	
	0.82	255	230 »	210 † 3		
22/III, 1922	0.78	245	230 inf.	230 inf.	215 inf.	0.82
	0.80	255	225 »	210 »	190 diseased	
	0.82	245	220 »	210 »	200 † 4½	
	0.84	245	215 »	210 † 3½		
27/III, 1922	0.78	240	240 inf.	230 inf.	240 inf.	0.82
	0.80	260	240 »	235 »	235 »	
	0.82	250	210 † 2			
	0.84	240	215 inf.	† 2½		

Washington Serum (received March 2nd, 1922).

	Toxin cc.	Weight of guinea-pigs gr.	2nd day gr.	3rd day gr.	4th day gr.	L.
2/III, 1922	0.80	230	230 inf.		225 inf.	0.82
	0.82	230	210 » † 2			
	0.84	230	210 »	† 2½		
	0.86	260	260 »	† 2½		
6/III, 1922	0.78	250	250 inf.	250 inf.	250 inf.	0.80
	0.80	255	230 »	215 »	† 3½	
	0.82	255	235 »	† 2½		

German Test Toxin ÷ Frankfort and Washington Standard Sera.

Frankfort Serum (same as above).

	Toxin cc.	Weight of guinea-pigs gr.	2nd day gr.	3rd day gr.	4th day gr.		L. † =
22/III, 1922	0.59	270	230 inf.	† 2½			<0.59
	0.61	245	205 »	190 diseased	† 3½		
	0.63	260	230 »	† 2½			
	0.65	245	† 1½				
27/III, 1922	0.55	260	225 inf.	200 inf.	185 inf.		0.57
	0.57	250	240 »	220 »	† 3½		
	0.59	240	200 » † 2				
	0.61	240	210 »	† 2½			
8/IV, 1922	0.55	270	250 inf.	250 inf.	240 inf.	† 5½	
	0.55	255	245 »	250 »	260 »	Still alive on the 10th day	
	0.56	245	240 »	250 »	240 »	† 7	
	0.56	250	225 »	240 »	250 »	Still alive on the 10th day	
	0.57	245	220 »	205 »	† 3½		
	0.57	240	220 »	210 »	190 diseased	† 10th day	
	0.58	260	225 »	215 »	† 3½		0.57
	0.58	245	210 »	190 »	† 3½		

Washington Serum (received March 2nd, 1922).

	Toxin cc.	Weight of guinea-pigs gr.	2nd day gr.	3rd day gr.	4th day gr.	L. † =
22/III, 1922	0.59	265	235 inf.	215 inf.	† 3½	<0.59
	0.61	265	225 »	† 2½		
	0.63	260	220 »	† 2½		
	0.65	245	195 »	† 2½		
27/III, 1922	0.55	260	250 inf.	240 inf.	240 inf.	0.57
	0.57	240	190 »	† 2½		

	Toxin cc	Weight of guinea-pigs gr.	2nd day gr.	3rd day gr.	4th day gr.		L $\frac{+}{+}$ =
	—	—	—	—	—		—
27/III, 1922 (*cont'd*)	0.59	260	240 »	240 inf.	$\frac{+}{+}$ 3½		
	0.61	240	210 »	$\frac{+}{+}$ 3			
	0.55	245	220 inf.	$\frac{+}{+}$ 2½			
	0.55	240	220 »	210 inf.	$\frac{+}{+}$ 3½		Not clear
	0.56	240	220 »	210 »	$\frac{+}{+}$ 3½		
	0.56	240	220 »	200 »	180 inf.	Still alive on the 10th day	
8/IV, 1922	0.57	240	210 »	205 »	190 »	$\frac{+}{+}$ 5½	
	0.57	250	215 »	200 »	$\frac{+}{+}$ 4		
	0.58	240	230 »	210 »	$\frac{+}{+}$ 3½		
	0.58	260	235 »	220 »	225 inf.	Still alive on the 10th day	

These experiments show clearly that L $\frac{+}{+}$ of both the test toxins examined is the same. The results obtained from the German and American units are identical. The reasonable conclusion is that the two units possess the same potency.

TETANUS ANTITOXIN.

A COMPARISON OF THE GERMAN, AMERICAN AND FRENCH ANTITOXIC UNITS.[1]

By Th. Madsen.

Based on experiments by L. E. Walbum.

For all these experiments the same test toxin was used. This toxin is four years old and is kept in a fluid state, covered with toluol at 2-4° C.

The toxins have been measured in such a way that mixtures of test toxin and test antitoxin brought to a total volume of 0.5-1.0 cc. have been left for one hour at 20° C. and then injected subcutaneously into the backs of mice weighing ca. 20 g. The animals were then kept under observation from 4 to 6 days.

1. Determination of L_0 with German Standard Serum.

The dried standard serum was dissolved in 0.9 % NaCl solution, 0.5 c.c. of this solution containing 0.001 antitoxic unit (A. U.). The toxin was diluted 1 + 9. The results were the following (Table I) :

In the tables following, abbreviations are used :

? = doubtful tetanus,
+ = mild tetanus,
+ + = moderate tetanus,
+ + + = severe tetanus,
+ +½+ + = very severe tetanus,
† = death.

Table I.

Test serum received	Dose of the Danish test toxin cc.	Date of test	Duration of observation in days					Result. L_0 =
			1	2	3	4	5	
21/III, 1922	0.032	3/V	0	0	0	0	0	0.034
	0.034		0	0	0	0	0	
	0.036		0	0	+	+	++	
	0.038		0	?	++	+++	++++	
	0.032	6/V	0	0	0	0	0	
	0.034		0	0	0	0	0	0.034
	0.036		0	0	0	+	+	
	0.038		0	0	+	+++	+++	

Table I (*continued*).

Test serum received	Dose of the Danish test toxin cc.	Date of test	Duration of observation in days					Result. L_o =
			1	2	3	4	5	
3/IV, 1917 Old test serum.	0.032	6/V	0	0	0	0	0	0.034
	0.034		0	0	0	0	0	
	0.036		0	0	0?	+	++	
	0.038		0	?	+	+++	+++	
21/III, 1922	0.032	10/V	0	0	0	0	0	0.034
	0.034		0	0	0	0	0	
	0.036		0	0	0	?	+	
	0.038		0	0	+	+	+	
1/VIII, 1922	0.036	15/VIII	0	0	0	0	0	0.039
	0.038		0	0	0	0	0	
	0.040		0	0	?	?	+	
	0.042		0	0	+	+	+++	
1/VIII, 1922	0.038	19/VIII	0	0	0	0	0	0.04
	0.040		0	0	0	0	?	
	0.042		0	?	+	++	+++	
	0.044		0	+	++	+++	++++	
19/VIII, 1922	0.038	31/VIII	0	0	0	0	0	0.04
	0.040		0	0	0	0	0	
	0.042		0	0	?	+	+	
	0.044		0	?	+	++	+++	
19/VIII, 1922	0.038	5/IX	0	0	0	0	0	0.04
	0.040		0	0	0	0	0	
	0.042		0	0	?	+	+	
	0.044		0	+	++	+++	+++	
19/VIII, 1922	0.038	9/IX	0	0	0	0	0	0.04
	0.040		0	0	0	0	0	
	0.042		0	0	?	+	+	
	0.044		0	+	++	+++	+	
19/VIII, 1922	0.038	13/IX	0	0	0	0	0	0.04
	0.040		0	0	0	0	0?	
	0.042		0	0	+	++	+++	
	0.044		0	0?	+++	++++	+	
19/VIII, 1922	0.038	10/X	0	0	0	0	0	0.04
	0.040		0	0	0	0	0	
	0.042		0	0	+	+	+++	
	0.044		0	?	++	+++	+++	
19/VIII, 1922	0.038	28/X	0	0	0	0	0	0.04
	0.040		0	0	0	0	0	
	0.042		0	0	+	+	+++	
	0.044		0	0	+	+	+++	

It appears from these experiments that the L_o value of our test toxin from May 3rd to August 19th, 1922, has changed from 0.034 cc. to 0.04 cc. Our experiments have shown that a fluid tetanus test toxin kept under toluol at 2-4° can be used for the testing of tetanus antitoxin just as well as a fluid diphtheria

toxin. In fact the deterioration which takes place in both of these two toxins is not an absolutely steady constant process but one which shows some irregularities.

2. Comparison between German and American Standard Sera

The dried standard serum was diluted in 0.9 % NaCl solution in such a quantity that 0.5 cc. of this solution would contain 0.001 A. U. The toxin was diluted 1 + 9.

The results were :

Table II.

American test-serum received	Dose of the Danish test-serum. L. cc.	Date of test	Amer. test serum unit.	Duration of observation in days 1	2	3	4	5	Result. 1 German unit = . . American units
26/IV. 1922	0.033	10/V	0.070	0	0	0	0	0	
			0.065	0	0	0	0	0	<65
			0.060	0	0	0	?	+	>60
			0.055	0	?	+	+	†	
26/IV. 1922	0.033	16/V	0.070	0	0	0	0	0	
			0.065	0	0	0	0	0	<65
			0.06	0	0	?	++	+++	>60
			0.055	0	?	+	++	+++	
30/VIII. 1922	0.04	31/VIII	0.070	0	0	0	0	0	
			0.068	0	0	0	0	0	68
			0.066	0	0	0?	++	+++	
			0.064	0	0	+	+++	†	
30/VIII. 1922	0.04	5/IX	0.070	0	0	0	0	0	
			0.068	0	0	0	0	0	68
			0.066	0	0	0?	++	++++	
			0.064	0	0	++	+++	++++	
30/VIII. 1922	0.04	9/IX	0.070	0	0	0	0	0	
			0.068	0	0	0	+	++	70
			0.066	0	0	+	+++	+++	
			0.064	0	0	+	+++	†	
30/VIII. 1922	0.04	18/IX	0.070	0	0	0	0	0	
			0.068	0	0	0	+	+	70
			0.066	0	0	+	+++	+++	
			0.064	0	+	+++	†		
30/VIII. 1922	0.04	17/X	0.070	0	0	0	0	—	
			0.068	0	0	+	+	+	70
			0.066	0	0	+	++	†	
			0.064	0	+	+++	†		
30/VIII. 1922	0.04	28/X	0.072	0	0	0	0	0	
			0.070	0	0	0	0	?	70
			0.068	0	0	?	+	+++	
			0.066	0	0?	+	+++	†	

From these experiments it appears that, while the first measurements (10/V and 16/V) gave 1 German A. U. = >60 and <65 American A. U., all the later determinations showed :

1 German A. U. = 68, 68, 70, 70, 70, 70, American A. U.

The first group of tests was performed with American standard serum which had been received on April 26th, 1922; the latter group with another American standard serum, which had been received August 30th, 1922.

3. Comparisons between German and French Standard Sera

The French standard serum, which is dried and pulverised, is said to contain 125 antitoxic units in 1 milligramme. This standard serum was not quite soluble; even after being kept four hours in a water bath at 38°, a little residuum was left.

The experiments gave the following results :

Table III.

Test serum received	Dose of the Danish test-toin. cc.	Date of test 1922	French test serum. mg.	Duration of observation in days					Result.
				1	2	3	4	5	
—	—	—	—	—	—	—	—	—	—
19/V, 1922	0.034	27/V	0.035	0	0	0	0	0	
			0.030	0	0	0	?	+	0.03 mg.
			0.025	0	?	++	±		Serum = 0.001
			0.020	0	++++	±			German unit
19/V. 1922	0.034	3/VI	0.035	0	0	0	0	0	0.03 mg.
			0.030	0	0	0	?	+	Serum = 0.001
			0.025	0	0?	+++	±		German unit
			0 020	0	±				

These two experiments showed in complete concordance that *1 German unit = ca. 3,800 French units.*

The result of all these experiments gave :

1 German unit = 69-70 American units = ca. 3,800 French units.

Remarks on the Different Methods of Measurements

(L_0 or L ±, mice or guinea-pigs).

The methods used in the different laboratories differ so much that a comparison seemed necessary. The object was to examine whether mice or guinea-pigs were the best to use, and whether L_0 or L ± would show the most constant results.

First of all, L_0 and L ± were determined by means of the German test serum, and after that a comparison between German and American test serum was performed with both these values.

Table IV.

L_0 determined on mice (limit : no tetanus on the fourth day).

German test serum.

Date of test 1922	Dose of the Dan. test toxin. cc.	Duration of observation in days 1	2	3	4	Result. L_0 =
—	—	—	—	—	—	—
9/IX	0.038	0	0	0	0	0.04
	0.040	0	0	0	0	
	0.042	0	0	?	+	
	0.044	0	+	++	+++	
13/IX	0.038	0	0	0	0	0.04
	0.040	0	0	0	0	
	0.042	0	0	+	++	
	0.044	0	0?	+++	+++	
10/X	0.038	0	0	0	0	0.04
	0.040	0	0	0	0	
	0.042	0	0	+	+	
	0.044	0	?	+++	+++	
28/X	0.038	0	0	0	0	0.04
	0.040	0	0	0	0	
	0.042	0	0	+	+	
	0.044	0	0	+	+	

Table V.

L_0 determined on guinea-pigs (limit : no tetanus on the fourth day).

German test serum.

Date of test 1922	Dose of the Dan. test toxin. cc.	Body weight	Duration of observation in days 1	2	3	4	Result. L_0 =
—	—	—	—	—	—	—	—
10/X	0.038	245	0	260 0	250 0	260 0	
	0.040	250	0	260 0	250 0	250 0?	0.04
	0.042	250	0	260 0	250 0	260 +	
	0.044	250	0	260 0	260 0?	260 ++	
28/X	0.038	250	0	230 0	240 0	240 0	
	0.040	250	0	250 0	250 0	240 0?	0.04
	0.042	240	0	230 0	240 0?	230 +++	
	0.044	245	0	240 0?	240 +	210 ++++	

Table VI.

$L\frac{+}{+}$ determined on mice (limit : death on the fourth day).

Date of test 1922	Dose of the Dan. test toxin. cc.	Duration of observation in days 1	2	3	4	Result. $L\frac{+}{+}$
10/X.	0.044	0	?	+++	+++	
	0.046	0	+	+++	+++	0.048
	0.048	0	+	++++	$\frac{+}{+}$	
	0.050	0	+++	$\frac{+}{+}$		
28/X.	0.044	0	0	+	+	
	0.046	0	0?	+++	++	0.048
	0.048	0	+	++++	$\frac{+}{+}$	
	0.050	0	++	$\frac{+}{+}$		

Table VII.

$L\frac{+}{+}$ determined on guinea-pigs (limit : death on the fourth day).

Date of test 1922	Dose of the Danish test toxin. cc.	Duration of observation in days 1	2	3	4	Result. $L\frac{+}{+}$ =
13/IX.	0.044	250 0	260 ?	260 ++	260 +++	
	0.046	250 0	260 +	260 +++	265 +++	0.048
	0.048	250 0	265 +	250++++	$\frac{+}{+}$	
	0.050	245 0	250+++	$\frac{+}{+}$		
10/X.	0.044	250 0	260 0	260 ?	260 ++	
	0.046	250 0	240 ?	250 +	260 ++	
	0.048	240 0	260 ?	250 +	$\frac{+}{+}$	0.048
	0.050	250 0	260 ?	250 +	250++++	$\frac{+}{+}$ 4½
	0.052	250 0	255 +	240 +++	$\frac{+}{+}$	
28/X.	0.044	245 0	250 ?	240 +	210++++	
	0.046	240 0	245 +	225++++	$\frac{+}{+}$	0.046
	0.048	240 0	220 +	215++++	$\frac{+}{+}$	
	0.050	250 0	235 +	230++++	220 $\frac{+}{+}$	$\frac{+}{+}$ 4½

Tested with these values, the relations between the strength of the German and American standard sera are valued as follows : (Tables VIII, IX, X and XI) :

Table VIII.

Test with $L_0 = 0.04$ c.c. in mice.

Date of test 1922	American units	Duration of observation in days				Result 1 German unit =
		1	2	3	4	
9/IX.	0.072	0	0	0	0	
	0.070	0	0	0	0	
	0.068	0	0	0?	−	70 American
	0.066	0	0	+	+++	
	0.064	0	0	+	− − −	
18/IX.	0.072	0	0	0	0	
	0.070	0	0	0	0	70 American
	0.068	0	0	0	+	
	0.066	0	0	+	− − −	
17/X.	0.072	0	0	0	0	
	0.070	0	0	0	0	70 American
	0.068	0	0	+	−	
	0.066	0	0	+	+++	
28/X.	0.072	0	0	0	0	
	0.070	0	0	0	0	70 American
	0.068	0	0	0?	+	
	0.066	0	0?	+	+++	

Table IX

Test with $L_0 = 0.04$ c.c. in guinea-pigs.

Date of test 1922	American units	Duration of observation in days				Result 1 German unit =
		1	2	3	4	
17/X.	0.080	250	250 0	225 0	270 0	
	0.078	240	235 0	210 0	260 0	
	0.076	240	235 0	210 0	240 0	<74 American?
	0.074	240	230 0	210 0?	240 ?	
28/X.	0.076	250	260 0	270 0	270 0	
	0.074	240	250 0	260 0	260 0	
	0.072	270	280 0	275 0	280 0	
	0.070	240	230 0	240 0	240 −	72 American
	0.068	250	260 0	250 0?	230 +	
	0.066	250	260 0	260 0?	240 ++	

Table X

Test with $L\frac{1}{1} = 0.048$ c.c. in mice.

Date of test 1922	American units	Duration of observation in days				Result 1 German unit =
		1	2	3	4	
17/X.	0.076	0	+	− −	?	
	0.074	0	+	?		
	0.072	0	++	?		>76 American
	0.070	0	?			

Table X (*continued*).

Date of test 1922	American units	Duration of observation in days 1	2	3	4	Result. 1 German unit =
28/X.	0.084	0	0	0	0	
	0.082	0	0	0	0?	80 American
	0.080	0	0	+	÷	
	0.078	0	0	+	+++	÷ 4½
	0.076	0	0	+++	÷	

Table XI.

Test with L ÷ = 0.048 c.c. in guinea-pigs.

Date of test 1922	American units	Duration of observation in days 1	2	3	4		Result. 1 German unit =
18. IX.	0.082	245 0	250 0	260 ?	250+++	÷ 6	
	0.080	250 0	270 0	270+	250 ÷		
	0.078	245 0	270 ?	270++	250 ÷		
	0.076	245 0	260 ?	270++	250 ÷		
	0.074	250 0	265 ?	270+++	270++++	† 4 1/2	80 American
	0.072	250 0	270 +	289+++	÷ 3½		
	0.070	250 0	270 +	260 ÷			
	0.068	245 0	260 +	250 ÷			
17. X.	0.088	250 0	250 0	230 0	270 0		
	0.086	240 0	235 0	220 0	240 ?		
	0.084	250 0	240 0	215 0	220+	÷ 6	
	0.082	250 0	245 0	225 0	240+++	† 4 1/2	
	0.080	250 0	250 0	235 ?	250+++	† 5 1/2	76-80 American
	0.078	250 0	240 ?	220+	220+++	† 4 1/2	
	0.076	240 0	235 ?	210+	225+++	† 4 1/2	
	0.074	250 0	250 0?	230+	260+++	† 4 1/2	
28. X.	0.084	250 0	260 0	255 0	250+		
	0.082	240 0	260 0	255+	230+++	÷ 5	
	0.080	240 0	240 0	240+	230+++	† 5 1/2	76-80 American
	0.078	260 0	250 0	260++	240++++	† 4 1/2	
	0.076	250 0	230 0	215+++	÷		

These experiments show the great difficulty of obtaining a clear determination with the L ÷ test; the limit is rather uncertain. As far as the L ÷ is concerned, the exact decision seems to be very difficult, the death of a tetanic animal within the critical four days depending on many different circumstances (weighing, handling, presence of other animals in the cage, etc.).

On the other hand, according to our experience, by using L_o and mice for the test, results of great constancy are obtained. This is due to the fact that in mice even slight traces of tetanus are easy

to observe, and here especially deviations of the tail act as a very fine indicator. In guinea-pigs, this decision is much more difficult and uncertain.

Résumé.

1 German unit corresponds to the following American units

	Mice	Guinea-pigs
	70	< 74
L_0	70	
	70	72
	70	
	> 76	80
$L \frac{1}{1}$	80?	76 — 80
		76 — 80

This résumé shows a remarkable difference in the tests with L_0 and $L \frac{1}{1}$, the German unit tested with L_0 being equal to ca. 70 American units both in the cases of mice and guinea-pigs, whilst tested with $L \frac{1}{1}$ it is much higher, about 76—80 American units.

This difference in the testing is rather remarkable and should be noted, especially when international comparisons are being dealt with.

It is desirable that this question should be examined and a uniform method agreed upon.

EXPERIMENTAL RESEARCHES INTO THE STANDARDISATION OF ANTI-DIPHTHERITIC AND ANTI-TETANIC SERA

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In accordance with the arrangements made at the London Conference in December 1921, the Committee which was instructed to study the anti-diphtheritic and anti-tetanic sera undertook to resume experiments regarding the various methods of testing these sera and to compare these methods, with the object of discovering the best scientific and practical standard on which an international agreement might be based.

In the present report I shall set forth the researches which have been carried out during 1922 in the Bacteriological Laboratory of Public Health of the Kingdom of Italy (1).

From a general standpoint, I would first of all point out that these researches may be divided into three series. The first deals with the methods employed to describe the potency of the respective toxins ; the second with the determination of the therapeutic power of sera; and the third with comparative tests to which the various methods have been submitted in order to discover their respective value and to decide upon the selection of one or other of them, taking into account their scientific reliability, their simplicity and the advantages presented by unity of standard and the facility with which animals suitable for the tests may be procured.

I. *Anti-Diphtheritic Serum.*

It is well known that there exists, in the case of this serum, an antitoxic unit which has been adopted throughout the world—the Ehrlich unit. Before the war the standardised Frankfort serum was adopted by most preparing and testing institutes. On the outbreak of the European War it became impossible for many countries to obtain from Frankfort the serum used as standard. Some

(1) Professors R. Maggiora and M. Pergola and Dr. L. Vivaldi took part in the experiments.

institutes therefore returned, as regards standardisation, to the old Behring method, while others abandoned their researches into antitoxic values and confined themselves to researches into sterility and the duration of efficacity, and the majority had recourse to the American standard serum prepared by the Central Laboratory of Hygiene of the United States.

At the London conference, however, it soon became apparent that there was no appreciable difference between the American unit and the Ehrlich unit, so that the work of the laboratories entrusted with technical revision was reduced to the verification of the value of the Washington unit in relation to the unit which was retained at the Frankfort Institute. As the preliminary researches confirmed the hypothesis of their equivalence, the subsequent accurate researches, instead of being directed towards the verification of the American method as compared with the German method, were confined to the application of the original Ehrlich method with standard toxins and sera of American and German origin, followed by a comparison of the results obtained.

With regard to the materials employed, we have pleasure in stating that, through the courtesy of Professor Madsen, Director of the Danish Sero-Therapeutic Institute, the American and German standard sera and the corresponding type toxins reached us directly from the Washington Office and the Frankfort Institute.

It is, I think, needless to go into details of the technique which was used, as this technique is absolutely identical in all tests and conforms entirely to the accepted Ehrlich method (1). I will merely add that, for purposes of information and control, several series have had to be recommenced, for, in view of the fact that this method is based on the power of resistance of animals to diphtheritic intoxication, and that this resistance is not—other things being equal —the same in all cases, we considered it necessary to repeat several series of experiments.

In the course of the comparative researches also, a large number of series of experiments was necessary to arrive at an accurate system of describing the potency of the standard toxins and sera from Germany and America. In Table I we have set out the most important series of experiments. The result of these experiments shows that, whether the standard serum and toxin used have the same origin, or whether the tests are "crossed", *i.e.*, when a serum and toxin of different origins are used, the results are almost identical; the few slight differences which may be observed are without practical importance.

It is also established that both sera—the American standard serum as well as the German serum—warrant perfect confidence in the test, provided that both standards are exactly dosed in relation to the Ehrlich antitoxic unit.

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(1) R. Otto and H. Hetsch, *Die staatliche Prüfung der Heilsera*. Jena, G. Fisher, 1921.

II. *Anti-Tetanic Serum.*

During the preliminary discussions at the London Conference, it was decided that it would be desirable to study the four best-known methods of testing the potency of anti-tetanic toxin—the German, American, French and Italian methods. We ourselves were engaged in special researches into the last-named, as its originator had completely changed the direction of his scientific researches.

As is well known, the Italian method is due to Dr. Tizzoni, who spent a considerable period in testing the action of anti-tetanic serum on the rabbit, following the principle, which is generally adopted, of neutralising the specific toxin. Since 1915, however, Tizzoni completely changed his views, and ended by utterly denying the value of this method, or of any other based on the same standard; he proposed to experiment with strychnine, and based his idea on the clinical antagonism which he believed to exist between that poison and tetanic anti-toxin.

This is not the place in which to discuss the scientific foundation of this new point of view. Those who are interested we would refer to Dr. Tizzoni's publications (1), but, in view of the author's reputation, we were bound to carry out extensive researches into this new process in order to determine its real practical value in the field of immunisation.

The technique of the new Tizzoni method, as described by the author, consists in the injection of 0.5-1 cubic centimetres of the serum under examination into the auricular vein of the rabbit (weight 1,200-1,500 grammes), and the further injection, after 24 hours, of the D.M.M. of sulphate or nitrate of strychnine, dosed at 0.6 milligrammes by the author, who advises that this dose should be corrected if necessary.

We had therefore to make numerous experiments in order to determine the D.M.M. of nitrate of strychnine. Moreover, even if great care is taken to use the purest products supplied by the best firms, the varying action of the poison on different subjects involves the sacrifice of a large number of animals, and consequently entails considerable expense. In point of fact, in order to produce the death of the rabbit in a sufficiently constant manner, we were obliged to make considerable alterations in the dose of 0.6 milligrammes per kilogramme adopted as D.M.M. by Tizzoni. In our experiments we have had to raise this dose to nearly double, *i.e.*, 1.10 milligrammes (Table II).

Finally, as is shown in Table III, the anti-tetanic serum exercises no protective action against strychnine poisoning, unless we use the fallacious criterion of a delay in the death of animals which

(1) G. Tizzoni and F. Perrucci, *Intorno ai criteri scientifici per giudicare in modo rapido e sicuro sulla reale efficacia del siero antitetanico* (*Il Policlinico*. Vol. XXII. M. 1915).

have been sero-therapeutically treated; and this result, according to witnesses, is not observed to be constant.

The same results are obtained if the guinea-pig (1) is used instead of the rabbit; and according to Tizzoni's latest report (see Table IV), the former is the more suitable animal.

We may therefore leave this subject, with the comment that we cannot accept this author's views. The value of the analogy between the symptoms of tetanus and those of strychnine is, we consider, purely clinical; and the results of an anti-strychnine action which he attributes to the anti-tetanic serum should, on the contrary, be attributed either to the use of sub-lethal doses of the poison or to the existence of protective substances other than the tetanus anti-toxin.

If we now turn to the methods based on the recognised principle of the evaluation of the protective power of serum immunised with respect to the corresponding specific toxin, we shall observe that the methods which are most usually employed in laboratories are the German and American methods. Both are based on Ehrlich's fundamental idea. The only differences between them are the difference in the unit of measurement and the difference in the kinds of animals the use of which is recommended for experiment. We originally followed the German method, but, for various reasons which will be set forth below, we have latterly adopted in our laboratory (testing section) the American method for anti-tetanic serum. We therefore found ourselves in an excellent position to contribute to the examination of the two antitoxic units by parallel tests. In order to ensure accuracy in our application of the German method, we used the standardised serum with the corresponding test toxin sent to us by Professor Kolle, Director of the Frankfort Institute of Experimental Therapeutics.

Following Kolle's instructions, the standard serum was dissolved in 17 cubic centimetres. Thus, one cubic centimetre corresponded exactly to 1 % of U.I. Behring. The test toxin was dissolved in 25 c.c.

In Sub-Table A of Table V we reproduce a series of tests which were faithfully carried out according to the regulations in force in the Reich (2).

Afterwards, in order to establish the relation between the German (Behring) and the American (Rosenau) antitoxic units, we used the same lot of serum which had been sent to us by the Frankfort Institute, at the same time testing in accordance with the American method as modified by Pergola (3).

As we did not receive a sufficient quantity of the German test toxin, we used one of our own dry toxins, after determining the D.M.M., which was 0.00006 grammes for a guinea-pig weighing

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(1) *Policlinico, Sez. Medica*, 1919, page 109.
(2) *Loc. cit.*
(3) M. Pergola, "Sull'accertamento dell'efficacia del siero anti-tetanico" (*Igiene Moderna*. Anno XI, No. 12. Dic. 1918).

350 gr.; 300 D.M.M. are therefore equivalent to 0.018 gr. After weighing out a certain quantity of toxin, we dissolved it in saline, in such a proportion that each c.c. should contain precisely 300 D.M.M., i.e. 0.018 gr. of toxin (1). By the Rosenau method, as technically modified by Pergola, the toxin anti-toxin mixture should be so prepared that the total volume should not exceed 3 c.c., and each c.c. should contain 100 D.M.M. plus one-tenth of U.I. Rosenau. To obtain this result, when the solution described above had been prepared, we dissolved the German standard-serum so that one-tenth of U. I. Rosenau should be contained in 0.5 c.c. of solution. Thus the three-tenths of U.I. Rosenau, which we took in accordance with the modified method, were contained in 1.5 c.c., which, together with 1 c.c. of toxin and 0.5 c.c. of saline solution, made up precisely the 3 c.c. required.

For each experiment we inoculated two guinea-pigs (weight 350 gr.) with 1 c.c. each of the mixture. The results obtained are set out in sub-table B of Table V.

We should, of course, have liked to extend our experiments much further, but, owing to the limited quantity of material which was sent to us, we were obliged to confine our work entirely to the fundamental point of the problem which was before us.

However, the tests which we were able to carry out are sufficient to justify the conclusion that the value of the American unit lies betwen 1/65 and 1/70 of the value of the German unit. A careful examination of the details of the experiments (Nos. 5, 6, 7 and 8 of sub-table B) with a consideration of the symptoms and the statistics of the survival of the animals, leads to the conclusion that this value is about 1/66.

As has already been observed, the Bacteriological Laboratory of Public Health formerly used the Behring method for the testing of anti-tetanic sera. During the war, however, it adopted the Rosenau method for the many experiments which were necessary; and this method presented notable practical advantages which led to its being definitely preferred.

These advantages are as follows :

(1) With the Rosenau method any good dry toxin can be used for testing, whereas the Behring method requires the test-toxin.

(2) Each lot is enough for two guinea-pigs, whereas the Behring method requires 8 mice.

(3) The preparation of the toxin anti-toxin mixture for inoculation is a much quicker process. With the Rosenau method only one mixture need be prepared for each lot, whereas the Behring method requires 8.

(4) Two guinea-pigs can be inoculated much more quickly than 8 mice.

(5) In general, the practical execution of the test is much simpler a matter, which is of particular importance when several

(1) Rosenau and Anderson, "The Standardisation of Tetanus Anti-toxin," *Hygiene Laboratory Bulletin*, No. 43, Washington. 1908.

lots have to be tested simultaneously. Thus for 10 lots we prepare 10 mixtures, and inoculate 20 guinea-pigs, whereas the Behring method requires the preparation of 88 mixtures, and the inoculation of 88 mice.

(6) It is much simpler and quicker to inoculate a guinea-pig with one cubic centimetre of fluid than a mouse with 0.4 c.c.

(7) As inoculation mixtures must remain in contact for a fixed time, when several lots are being tested simultaneously, several persons must be employed simultaneously in inoculating mice; otherwise, in the mixtures used at the end of a series of inoculations, the component parts will have remained in contact longer than they should. A single experienced person, however, takes very little time to inoculate 20 guinea-pigs.

(8) We find it much easier to obtain guinea-pigs (which are bred by many private individuals) than mice.

(9) The guinea-pig is less delicate and more easily handled.

(10) From the point of view of expense, there is probably no great difference between the use of mice and of guinea-pigs, in view of the fact that 4 times as many mice are used.

Table I.

Control Test on Kolle and American Anti-diphtheria Standard Serum

Toxin	Kolle Standard serum				American standard serum				
L†	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	
—	—	—	—	—	—	—	—	—	
0.61			+					+	
0.60			+					+	
0.59				+				+	
0.58				+				+	
0.57									Series A
0.56									
0.61			+			+			
0.60							+		
0.59							+		
0.58									Series B
0.57									
0.56									
0.61				+			+		
0.60						+			
0.59						+			
0.58									Series C
0.57									
0.56									
0.61				+			+		
0.60								+	
0.59								+	Series D
0.58									

Table I (*continued*)

Toxin	Kolle Standard serum				American standard serum				
L†	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	
—	—	—	—	—	—	—	—	—	
0.61			+	−				+	
0.61				+			+		Series E
0.61				+				+	
0.61				+			+		
0.60								+	
0.60									
0.60								+	
0.60							+		
0.59				+					
0.59									
0.59								+	
0.59									
0.58									
0.58									
0.58									
0.58									

α † 0.61
α ○ 0.60

Table II.

D.M.M. DETERMINED IN RABBITS (NITRATE OF STRYCHNINE)

Numerical indication	Animal used	Weight in gr.	Dose of nitrate of strychnine inoculated mmgr.	Result
—	—	—	—	—
1	Rabbit	1,170	0.5 p. kg.	survived
2	»	1,570	» »	»
3	»	1,330	» »	»
4	»	1,280	» »	»
5	»	1,660	» »	»
6	»	1,610	» »	»
7	»	1,530	» »	»
8	»	1,330	0.6 »	»
9	»	1,480	» »	»
10	»	1,670	» »	»
11	»	1,430	» »	»
12	»	1,000	» »	† in 20′
13	»	1,800	7 »	survived
14	»	1,800	» »	»
15	»	1,650	» »	»
16	»	1,700	» »	† in 20′
17	»	1,620	0.75 »	survived
18	»	1,340	» »	»
19	»	1,370	0.8 »	»
20	»	1,520	» »	† in 25′
21	»	1,450	1.0 »	survived
22	»	1,450	» »	† in 35′
23	»	1,760	» »	† in 1 hour
24	»	1,600	1.05 »	survived
25	»	1,650	1.10 »	† in 40′
26	»	1,600	» »	† in 22′
27	»	1,600	» »	† in 35′
28	»	1,050	» »	† in 20′

Table III.

CONTROL OF THE TIZZONI METHOD ON RABBITS.
SUBCUTANEOUS INOCULATION OF ANTI-TETANIC SERUM AND STRYCHNINE IN DIFFERENT SEATS OF INOCULATION

Numerical Indication	Animal used	Weight in gr.	Dose of serum injected cc.	Dose of nitrate of strychnine injected mmgr.	Interval after injection	Result	
1	rabbit	1,100	. . . .	1.21	. . . .	½ in 14′	Control of the D. M. M. (mmgr 10 par kg.).
2	»	1,210	. . . .	1.33	. . . .	½ » 47′	
3	»	1,110	. . . .	1.22	. . . .	½ » 10′	
4	»	1,600	. . . .	1.76	. . . .	½ » 35′	
5	rabbit	1,650	0.5	1.815	24 h.	½ in 50′	Serum of the Serotherapeutic Institute at Milan.
6	»	1,900	1.0	2.09	»	½ » 20′	
7	»	1,350	1.0	1.48	»	½ » 1 h.	
8	»	1,150	1.0	1.26	»	½ » 17′	
9	»	1,300	0.5	1.43	»	½ » 30′	
10	rabbit	1,700	0.5	1.87	24 h.	½ in 21′	Serum of the Institute of Tizzoni.
11	»	1,800	1.0	1.98	»	½ » 1 h.	
12	»	1,200	1.0	1.32	»	½ » 50′	
13	»	1,370	0.5	1.50	»	½ » 52′	
14	»	1,320	0.5	1.45	»	½ » 1 h. 8′	

Table IV.

CONTROL OF THE TIZZONI METHOD ON GUINEA-PIGS.
SUBCUTANEOUS INOCULATION OF ANTI-TETANIC SERUM AND STRYCHNINE IN DIFFERENT SEATS OF INOCULATION

Numerical Indication	Animal used	Weight in gr.	Dose of serum injected cc.	Dose of strychnine injected mmgr.	Time-interval after injection	Result	
1	guinea-pig	420	. . . . .	1.68	. . . .	½ in 35′	Control of D. M. M. (4 mmgr par kg.).
2	»	520	. . . . .	2.08	. . . .	. . . . .	
3	»	310	. . . . .	1.24	. . . .	. . . . .	
4	»	480	. . . . .	1.92	. . . .	. . . . .	
5	»	320	. . . . .	1.28	. . . .	½ in 30′	
6	»	300	. . . . .	1.20	. . . .	½ » 40′	
7	»	280	. . . . .	1.12	. . . .	. . . . .	
8	»	270	. . . . .	1.08	. . . .	½ in 18′	
9	»	250	. . . . .	1.00	. . . .	. . . . .	
10	»	250	. . . . .	1.00	. . . .	½ in 22′	
11	guinea-pig	450	0.5	1.08	24 h.	½ in 6′	Serum of the Tizzoni Institute.
12	»	330	»	1.32	»	. . . . .	
13	»	420	»	1.68	»	. . . . .	
14	»	330	»	1.32	»	. . . . .	
15	»	250	»	1.00	»	½ in 12′	
16	»	250	»	1.00	»	½ » 12′	
17	»	220	»	0.88	»	½ » 27′	
18	»	230	»	0.92	»	. . . . .	

Table IV (*continued*)

Numerical Indication	Animal used	Weight in gr.	Dose of serum injected cc.	Dose of strychnine injected mmgr.	Time-interval after injection	Result	
—	—	—	—	—	—		
19	Guinea-pig	550	0.5	2.20	24 h.	. . . . .	Serum of the Serotherapeutic Institute at Milan.
20	»	370	»	1.48	»	† in 41′	
21	»	450	»	1.80	»	. . . . .	
22	»	330	»	1.32	»	† in 15′	
23	»	250	»	1.00	»	. . . . .	
24	»	250	»	1.00	»	. . . . .	
25	»	280	»	1.12	»	. . . . .	
26	»	250	»	1.00	»	. . . . .	

Table V.

CONTROL OF THE ANTI-TETANIC SERUM CARRIED OUT ON JUNE 21ST, AT NOON

Table A

Behring's method	Animal inoculated	Dose of toxin inoculated	Dates 22. VI	23	24	25	26	27
—	Mouse	—	—	—	—	—	—	—
	1	0,08	—	—	—	—	—	—
	2	0,09	—	—	○	○	○	○
	3	0,10	—	○	○	◔	◔	◔
	4	0,11	○	○	◔	◔	◔	◔
	5	0,12	◔	◔	◓	◓	⊕	●
	6	0,13	◓	◓	◓	⊕	●	●
	7	0,14	◓	⊕	●	●	†	
	8	0,15	⊕	●	†			

Table B

Rosenau's method modified by Pergola	Animal inoculated	Relationship between the Rosenau and Behring unit	Dates 22. VI	23	24	25	26	27	
	Guinea-pig								
	1	1/50	—	—	—	—	—	—	
	2		—	—	—	—	—	—	
	3	1/60	—	—	—	—	—	—	
	4		—	—	—	—	—	—	
	5	1/65	◔	⊕	●	†			found dead on the 25. VI at 15 h.
	6		◔	⊕	●	●	†		
	7	1/70	◓	●	●	†			found dead in the morning of the 26th day.
	8		◓	●	●	†			
	9	1/80	⊕	●	†				found dead in the morning of the 25th (before noon).
	10		⊕	●	†				

Explanation
- — no symptom.
- ○ slight trace.
- ◔ slight symptoms.
- ◓ distinct symptoms.
- ⊕ fairly strong symptoms.
- ● strong symtoms.
- † deaths.

Dilution for reduction of the German unit to the American unit.

	Serum		Saline
1/50	= 1 cc.	+	1.50 cc.
1/60	= 1 cc.	+	2 cc.
1/65	= 1 cc.	+	2.25 cc.
1/70	= 1 cc.	+	2.50 cc.
1/80	= 1 cc.	+	3 cc.

RESEARCHES WITH REGARD TO TETANUS ANTITOXIN

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The comparative experiments to determine the potency of the various anti-tetanic sera and the value of the standards employed in America and Germany for estimating potency were conducted in such a manner that one set of experiments was devoted to the titration of American standard serum by means of Frankfort test toxin on the German method. Details are given in Tables I, IIa, IIb, IIIa, IIIb and IIIc.

The experiments were carried out in the following manner :

One cc. of American standard serum was first of all dissolved in 7.5 cc. of saline for the purpose of obtaining a first approximation as to the degree of its strength (Table I).

Subsequently, dilutions of the standard serum were made in 6.5 cc. and 5 cc. of salt solution, and at the same time the Frankfort standard serum was tested for the purpose of comparison.

Doses of the Frankfort standard serum increasing from 0.08 to 0.15 were employed as toxin doses. Mixing, titration and injection were carried out in accordance with German official instructions.

The experiment with the dilution 1+6.5 NaCl was repeated twice (Tables IIa, IIb), and the experiment with the serum dilution 1+5 NaCl was repeated three times (Tables IIIa, IIIb, IIIc).

Result : The American standard serum diluted with 5 cc. of saline contains 1/100 of the German antitoxin unit per ccm. This corresponds to the dilution prescribed for the German standard serum.

In a second series of experiments, the Frankfort standard serum was titrated by the German method by means of American standard toxin. In each experiment the American standard toxin was employed in a dilution of 1/100, 1/135, 1/140, 1/150 and 1/200, and, after being mixed with the German standard serum in accordance with the German method, was injected into the mice. In this series of experiments, as in the previous series, the same standard toxin (in the first case the American standard serum, in the second case the Frankfort standard serum) was always employed in equal doses with the corresponding toxin, and therefore German standard serum was employed with German test toxin and American standard serum with American test toxin. These experiments showed that 0.078 of the American standard toxin, diluted in the ratio of 1 to 140, and 0.14 of the Frankfort standard toxin diluted in the ratio of 1 to 100, were neutralised by an equal number of antitoxin units. These doses of the respective toxins, when mixed with 1/1000 of German a. u., produce the same disease

developments and have the same lethal potency in the case of mice into which these mixtures have been injected.

The experiment was carried out in all details strictly according to the procedure for such experiments published in the work of Otto and Hetsch (Work at the National Institute for Experimental Therapeutics and the Georg Speyer Institute, 1921, Vol. XIII, published by Fischer, Jena) which gives precise and detailed information with regard to the testing of this serum. All the particulars are to be obtained from the examples given in this work.

First of all, a standard serum tube is carefully opened by filing; then four or five cc. of a solution of common salt is poured from a graduated pipette on to the serum powder, which is contained in the tube and which, according to instructions, should be dissolved in a solution of 17 cc. of common salt, and thereafter the tube is sealed again with a burnt cork. If, after repeated shaking, the serum is completely dissolved, the solution is then poured off into a sterilised phial and the tube with the remaining 13 or 12 cc. of the solution of common salt is thoroughly rinsed so that no traces of the serum are left in it. The phial then contains 17 cc. of a solution of the standard serum and each cc. of this solution contains 1/100 I. U.

In precisely the same way, after opening a toxin tube, 27 cc. of test toxin solution are prepared from its contents.

The serum which is to be tested—after previously being tested for sterility—is diluted in accordance with the titre supplied by the factory, so that one cubic centimetre of the dilution must contain exactly 1/100 I. U. When this has been done, the mixtures of toxin and serum are placed *in vitro*. For this purpose two sets of phials are required, each set numbering eight. One cc. of standard serum solution, carefully measured with a scientifically accurate pipette, is introduced into each of the phials of the first set and one cc. of the solution prepared from the serum to be tested into each of the phials of the second set.

Increasing doses of toxin—in the case of the present toxin the doses are from 0.8 to 1.5—are then added to each set, and also sufficient saline to bring up the total contents of each phial to 4 cc. For practical reasons, saline is poured in first, the serum dilution is added and, finally, the toxin solution.

The phials then contain the following :

Tube	Standard serum solution	Toxin solution	Saline	Tube	Dilution of the serum to be tested	Toxin solution	Saline.
1	1 cc.	0.8 cc.	2.2 cc.	9	1 cc.	0.8 cc.	2.2 cc.
2	»	0.9 »	2.1 »	10	»	0.9 »	2.1 »
3	»	1.0 »	2.0 »	11	»	1.0 »	2.0 »
4	»	1.1 »	1.9 »	12	»	1.1 »	1.9 »
5	»	1.2 »	1.8 »	13	»	1.2 »	1.8 »
6	»	1.3 »	1.7 »	14	»	1.3 »	1.7 »
7	»	1.4 »	1.6 »	15	»	1.4 »	1.6 »
8	»	1.5 »	1.5 »	16	»	1.5 »	1.5 »

The phials, carefully corked, remain for half-an-hour at the temperature of the room, but shielded from the daylight by tin-plate covers.

Then two groups, each consisting of eight white mice of absolutely equal weight (15 gr.), are infected with the mixtures in the phials, each mouse being given 0.4 cc. of a single mixture subcutaneously, under the skin of the right hind foot, and therefore receiving 1/1000 I U + increasingly large doses (0.08 to 0.15) of toxin solution.

The animals are kept under observation for a period of six days. The degree of tetanus ("traces", "distinct", "strong", etc., see Table), which can be definitely ascertained, is accurately recorded every day. Experience enables the observer to distinguish very clearly the extent of the toxic action by the degree of the curvature shown by the mice when held up by the tail. If the serum which is being tested is up to the given standard of strength, the results of the experiments must coincide exactly in the case of both groups of animals. If, however, the serum is weaker than is stated, the results supervene after a proportionate delay and from this delay it is easy to determine the extent to which the serum in question falls short of the standard of strength assigned it by the factory.

The tables show the successive stages of two experiments of this kind to determine potency.

Determination of the Potency of the Anti-Tetanic Serum on July 19th

Potency as given by factory : 4 I U in 1 cc.

	Standard serum	Serum X	Standard serum	Serum X
	Dose 0.08		*Dose 0.12*	
	Mouse 1	Mouse 9	Mouse 5	Mouse 13
20.7	0	0	traces	faint traces
21.7	0	0	slight	traces
22.7	0	0	slight-distinct	slight
23.7	0	0	distinct	slight-distinct
24.7	0	0	fairly strong	distinct
25.7	0	0	strong	fairly strong
	Dose 0.09		*Dose 0.13*	
	Mouse 2	Mouse 10	Mouse 6	Mouse 14
20.7	?	0	slight-distinct	traces
21.7	faint traces	?	»	slight
22.7	»	faint traces	distinct	slight-distinct
23.7	»	faint traces	fairly strong	distinct
24.7	»	»	strong	fairly strong
25.7	»	»	+ 6	strong
	Dose 0.1		*Dose 0.14*	
	Mouse 3	Mouse 11	Mouse 7	Mouse 15
20.7	faint traces	?	slight-distinct	slight
21.7	»	faint traces	distinct	distinct
22.7	traces	»	fairly strong	fairly strong
23.7	»	traces	strong	strong
24.7	»	»	+ 5	+ 5
25.7	»	»	.	.
	Dose 0.11		*Dose 0.15*	
	Mouse 4	Mouse 12	Mouse 8	Mouse 16
20.7	faint traces	faint traces	distinct	distinct
21.7	traces	»	fairly strong	fairly strong
22.7	»	traces	strong	strong
23.7	slight	»	+ 4	+ 4
24.7	»	»	.	.
25.7	»	slight	.	.

Result : admitted on July 26th to be "four times".

Determination of the Potency of the Anti-Tetanic Serum on September 26th

Potency as given by factory : 6 I U in 1 cc.

	Standard serum	Serum Y	Standard serum	Serum Y
	Dose 0.08		*Dose 0.12*	
	Mouse 1	Mouse 9	Mouse 5	Mouse 13
27.9	0	faint traces	traces	distinct
28.9	0	traces	slight	fairly strong
29.9	0	»	slight-distinct	strong
30.9	0	slight	distinct	+ 4
1.10	0	slight-distinct	»	.
2.10	0	distinct	fairly strong	.
	Dose 0.09		*Dose 0.13*	
	Mouse 2	Mouse 10	Mouse 6	Mouse 14
27.9	?	traces	slight	fairly strong
28.9	faint traces	slight	slight-distinct	strong
29.9	»	slight-distinct	distinct	+ 3
30.9	—	distinct	fairly strong	.
1.10	»	fairly strong	strong	.
2.10	»	strong	+ 5	.
	Dose 0.1		*Dose 0.14*	
	Mouse 3	Mouse 11	Mouse 7	Mouse 15
27.9	faint traces	traces-slight	slight	strong
28.9	»	slight-distinct	distinct	fatal
29.9	traces	distinct	fairly strong	+ 2 1/4
30.9	»	fairly strong	»	.
1.10	»	strong	+ 5	.
2.10	slight	+ 6.	.	.
	Dose 0.11		*Dose 0.15*	
	Mouse 4	Mouse 12	Mouse 8	Mouse 16
27.9	faint traces	slight-distinct	distinct	+ 1
28.9	traces	distinct	fairly strong	.
29.9	»	fairly strong	strong	.
30.9	slight	strong	+ 4	.
1.10	»	+ 5	.	.
2.10	»	.	.	.

Result : admitted on October 3rd to be "four times".

A few additional experiments were also carried out to determine the potency of the American standard serum with the American test toxin in accordance with the rules for measuring the potency of anti-tetanic serum laid down by Rosenau and Anderson. These rules are as follow :

The "immunity unit" for the determination of the antitoxin is

taken as ten times the minimum quantity of anti-tetanic serum which will keep a guinea pig, weighing 350 grammes and infected with the official test dose of test toxin, alive for a period of four days. The test dose consists of rather less than 100 lethal doses of test toxin (guinea pig, 350 gr.); *i.e.* $\frac{1}{10}$ I.U., mixed with 100 such lethal doses, contains exactly enough free toxin to kill the animal in four days.

The potency of an unknown serum is measured indirectly by means of the toxin, the 4 + potency being employed as the test potency. The 4 + dose is the smallest quantity of toxin which neutralises $\frac{1}{10}$ of I. U. + a quantity of toxin which is sufficient to kill a guinea pig in four days.

Diminishing quantities of 1,000 volume antitoxin solution (1cc—0.001) are mixed with 1 cc. of the test toxin dose and, after standing for a period of one hour at the temperature of the room, are injected subcutaneously at a volume of 4 cc. of fluid, into the abdominal region of a guinea-pig weighing 350 gr., approximately at the level of the navel. The number of the I. U. contained in the serum is obtained from the quantity of antitoxin which maintains the animal alive for four days.

Example : Test toxin contains 100 minimum lethal doses in 0.0006. About 20 to 25 milligrammes are weighed out on a weighing glass the weight of which has been ascertained by scaling. The toxin is dissolved in the glasses in a solution of common salt in the ratio of 0.1 dry toxin to 166.66 cc. NaCl solution. Each single cc. of the solution then contains 0.0006 of the original toxin.

0.0006 Toxin + 0.001 cc. 1.000 volumes antitoxin solution : † 2 days 4. hrs.

0.0006 Toxin + 0.0015 cc. 1,000 volume antitoxin solution : † 4 days 1 hr.

0.0006 Toxin + 0.002 cc. 1,000 volume antitoxin solution : symptoms.

0.0006 Toxin + 0.0025 cc. 1,000 volume antitoxin solution : slight symptoms.

0.0006 Toxin + 0.003 cc. 1,000 volume antitoxin solution : 0.

0.0015 contained $\frac{1}{10}$ American I. U.

The serum therefore contains 66 I. U. One German I. U. about 66 American I. U.

This experiment proves that one unit of the German anti-tetanic serum contains 65-66 American units.

The shortage of animals, however, prevented these experiments from being carried through, but they are being continued. The foregoing facts show that, whether the German or American anti-tetanic serum or toxin is employed, the ratio between the units of the two standard sera may also be successfully determined with sufficient accuracy by experiments on white mice.

Protocol I.

Testing of American standard serum through Frankfort test toxin on mice.

Serum Dilution 1 + 7.5 NaCl

Mouse 1 : 0.08 Toxin	Mouse 2 : 0.09 Toxin	Mouse 3 : 0.1 Toxin	Mouse 4 : 0.11 Toxin
31.5 0	slight trace	trace	slight
1.6 slight trace	trace	slight	slight-dist.
2.6 »	slight	slight-dist.	distinct
3.6 »	»	distinct	»
4.6 »	»	»	fairly strong
5.6 »	»	»	strong

Mouse 5 : 0.12 Toxin	Mouse 6 : 0.13 Toxin	Mouse 7 : 0.14 Toxin	Mouse 8 : 0.15 Toxin
31.5 slight-dist.	distinct	fairly strong	strong
1.6 fairly strong	fairly strong	strong	+ 2
2.6 strong	strong (dying)	+ 3	
3.6 + 4	+ 3¼		

Protocol II.

Testing of American standard serum against standard Frankfort toxin on mice.

Serum Dilution 1 + 6.5 NaCl

	German Standard Serum	American Standard Serum		German Standard Serum	American Standard Serum
	0.08 Toxin.	*0.08 Toxin.*		*0.08 Toxin.*	*0.08 Toxin.*
3.6	0	0	14.6	0	?
4.6	0	0	15.6	0	slight trace
5.6	0	0	16.6	0	»
6.6	0	?	17.6	0	»
7.6	0	slight trace	18.6	0	»
			19.6	0	»
	0.09 Toxin.	*0.09 Toxin.*		*0.09 Toxin.*	*0.09 Toxin.*
3.6	?	?	14.6	?	slight trace
4.6	slight trace	slight trace	15.6	slight trace	trace
5.6	»	»	16.6	»	»
6.6	»	»	17.6	»	»
7.6	»	trace	18.6	»	»
			19.6	»	»
	0.1 Toxin.	*0.1 Toxin.*		*0.1 Toxin.*	*0.1 Toxin.*
3.6	slight trace	slight trace	14.6	slight trace	trace
4.6	trace	trace	15.6	»	trace-slight
5.6	»	»	16.6	»	slight
6.6	slight	slight	17.6	trace	»
7.6	»	slight-dist.	18.6	»	slight-dist.
			19.6	»	»

Protocol II (*continued*)

	German Standard Serum	American Standard Serum		German Standard Serum	American Standard Serum
	0.11 Toxin.	*0.11 Toxin.*		*0.11 Toxin.*	*0.11 Toxin.*
3.6	slight trace	trace	14.6	slight trace	slight
4.6	trace	slight	15.6	trace	slight-dist.
5.6	slight	slight-distinct	16.6	»	»
6.6	»	distinct	17.6	slight	distinct
7.6	»	fairly strong	18.6	»	fairly strong
			19.6	»	strong
	0.12 Toxin.	*0.12 Toxin.*		*0.12 Toxin.*	*0.12 Toxin.*
3.6	trace	slight	14.6	trace	slight-dist.
4.6	slight	distinct	15.6	slight	distinct
5.6	distinct	fairly strong	16.6	slight-dist.	fairly strong
6.6	fairly strong	strong	17.6	distinct	† 4
7.6	»	† 5	18.6	fairly strong	
			19.6	strong	
	0.13 Toxin.	*0.13 Toxin.*		*0.13 Toxin.*	*0.13 Toxin.*
3.6	slight	fairly strong	14.6	slight	distinct
4.6	distinct	strong	15.6	slight-dist.	strong
5.6	fairly strong	† 3	16.6	distinct	† 3
6.6	strong		17.6	fairly strong	
7.6	»		18.6	strong	
			19.6	† 6	
	0.14 Toxin.	*0.14 Toxin.*		*0.14 Toxin.*	*0.14 Toxin.*
3.6	distinct	strong	14.6	slight-dist.	fairly strong
4.6	fairly strong	dying	15.6	distinct	† 2
5.6	strong	† 2¼	16.6	fairly strong	
6.6	† 4		17.6	strong	
			18.6	† 5	
	0.15 Toxin.	*0.15 Toxin.*		*0.15 Toxin.*	*0.15 Toxin.*
3.6	fairly strong	strong	14.6	distinct	strong
4.6	strong	† 2	15.6	fairly strong	† 1½
5.6	† 3		16.6	strong	
			17.6	»	
			18.6	† 4½	

Protocol III.

Testing of American standard serum through standard Frankfort toxin on mice.

Serum Dilution 1 + 5 NaCl

III a			III b			III c		
	German Standard Serum	American Standard Serum		German Standard Serum	American Standard Serum		German Standard Serum	American Standard Serum
	0.08 Toxin.	*0.08 Toxin.*		*0.08 Toxin.*	*0.08 Toxin.*		*0.08 Toxin.*	*0.08 Toxin.*
9.6	0	0	14.6	0	0	24.6	0	0
10.6	0	0	15.6	0	0	25.6	0	0
11.6	0	0	16.6	0	0	26.6	0	0
12.6	0	0	17.6	0	0	27.6	0	0
13.6	0	0	18.6	0	0	28.6	0	0
14.6	0	0	19.6	0	0	29.6	0	0
	0.09 Toxin.	*0.09 Toxin.*		*0.09 Toxin.*	*0.09 Toxin.*		*0.09 Toxin.*	*0.09 Toxin.*
9.6	0	0	14.6	?	?	24.6	?	?
10.6	?	?	15.6	slight trace	slight trace	25.6	slight trace	slight trace
11.6	slight trace	slight trace	16.6	»	»	26.6	»	»
12.6	»	»	17.6	»	»	27.6	»	»
13.6	»	»	18.6	»	»	28.6	»	»
14.6	»	»	19.6	»	»	29.6	»	»
	0.1 Toxin.	*0.1 Toxin.*		*0.1 Toxin.*	*0.1 Toxin.*		*0.1 Toxin.*	*0.1 Toxin.*
9.6	?	?	14.6	slight trace	slight trace	24.6	slight trace	slight trace
10.6	slight trace	slight trace	15.6	»	»	25.6	»	»
11.6	»	»	16.6	trace	trace	26.6	trace	trace
12.6	trace	trace	17.6	»	»	27.6	»	»
13.6	»	»	18.6	»	»	28.6	»	»
14.6	»	»	19.6	»	»	29.6	»	»
	0.11 Toxin.	*0.11 Toxin.*		*0.11 Toxin.*	*0.11 Toxin.*		*0.11 Toxin.*	*0.11 Toxin.*
9.6	slight trace	slight trace	14.6	slight trace	slight trace	24.6	slight trace	slight trace
10.6	trace	trace	15.6	trace	trace	25.6	trace	trace
11.6	»	»	16.6	»	»	26.6	slight	slight
12.6	slight	slight	17.6	slight	slight	27.6	»	»
13.6	»	»	18.6	»	»	28.6	»	»
14.6	»	»	19.6	»	»	29.6	»	»

	III a		III b		III c	
	German Standard Serum	American Standard Serum	German Standard Serum	American Standard Serum	German Standard Serum	American Standard Serum
	0.12 Toxin.	*0.12 Toxin.*	*0.12 Toxin.*	*0.12 Toxin.*	*0.12 Toxin.*	*0.12 Toxin.*
9.6	trace	trace	14.6 trace	trace	24.6 trace	trace
10.6	slight	slight	15.6 slight	slight	25.6 slight	slight
11.6	slight-dist.	slight-dist.	16.6 slight-dist.	slight-dist.	26.6 slight-dist.	slight-dist.
12.6	distinct	distinct	17.6 distinct	distinct	27.6 distinct	distinct
13.6	fairly strong	fairly strong	18.6 fairly strong	fairly strong	28.6 »	»
14.6	strong	strong	19.6 strong	strong	29.6 »	»
	0.13 Toxin.	*0.13 Toxin.*	*0.13 Toxin.*	*0.13 Toxin.*	*0.13 Toxin.*	*0.13 Toxin.*
9.6	slight	slight	14.6 slight	slight	24.6 slight	slight
10.6	slight-dist.	slight-dist.	15.6 slight-dist.	slight-dist.	25.6 slight-dist.	slight-dist.
11.6	distinct	distinct	16.6 distinct	distinct	26.6 distinct	distinct
12.6	fairly strong	fairly strong	17.6 fairly strong	fairly strong	27.6 fairly strong	fairly strong
13.6	strong	strong	18.6 strong	strong	28.6 strong	strong
14.6	† 6	† 6	19.6 † 6	† 6	29.6 † 6	† 6
	0.14 Toxin.	*0.14 Toxin.*	*0.14 Toxin.*	*0.14 Toxin.*	*0.14 Toxin.*	*0.14 Toxin.*
9.6	slight-dist.	slight-dist.	14.6 slight-dist.	slight-dist.	24.6 slight-dist.	slight-dist.
10.6	distinct	distinct	15.6 distinct	distinct	25.6 distinct.	distinct
11.6	fairly strong	fairly strong	16.6 fairly strong	fairly strong	26.6 fairly strong	fairly strong
12.6	strong	strong	17.6 strong	strong	27.6 strong	† 4
13.6	† 5	† 5	18.6 † 5	† 5	28.6 † 5	
14.6						
	0.15 Toxin.	*0.15 Toxin.*	*0.15 Toxin.*	*0.15 Toxin.*	*0.15 Toxin.*	*0.15 Toxin.*
9.6	distinct	distinct	14.6 distinct	distinct	24.6 distinct	distinct
10.6	fairly strong	fairly strong	15.6 fairly strong	fairly strong	25.6 strong	strong
11.6	strong	strong	16.6 strong	strong	26.6 † 3	† 3
12.6	† 4	† 4	17.6 dying † 4½	† 4		

TREASURY DEPARTMENT UNITED STATES
PUBLIC HEALTH SERVICE
HYGIENIC LABORATORY, WASHINGTON

DIRECTOR G. W. McCOY

Protocol covering comparative potency tests on the German and the American Diphtheria Antitoxin Units and on the German and the American Tetanus Antitoxin Units.

(*a*) COMPARATIVE POTENCY TEST ON GERMAN AND AMERICAN DIPHTHERIA ANTITOXIN UNITS

No. of pigs	Average wt. gms.	Toxin	Dose c.c.	Antitoxin	Dose	Survived days-hours (average)
4	260	American M 266 C	0.255	American B 178	1 i. u.	4 18
4	260	»	0.265	»	1 i. u.	2 22
4	262	»	0.275	»	1 i. u.	2 13
3	257	German no number	0.58	»	1 i. u.	3 17
3	260	»	0.61	»	1 i. u.	3 17
4	265	»	0.64	»	1 i. u.	2 6
4	265	American M 266 C	0.255	American B 181	1 i. u.	4 21
4	265	»	0.265	»	1 i. u.	3 17
4	270	»	0.275	»	1 i. u.	2 12
3	263	German no number	0.58	»	1 i. u.	3 17
3	266	»	0.61	»	1 i. u.	3 3
4	265	»	0.64	»	1 i. u.	2 12
4	265	American M 266 C	0.255	German no number	1 i. u.	6 1
4	270	»	0.265	»	1 i. u.	4 18
4	268	»	0.275	»	1 i. u.	2 23
3	266	German no number	0.58	»	1 i. u.	5 4
3	273	»	0.61	»	1 i. u.	3 7
4	270	»	0.64	»	1 i. u.	2 21

(*b*) COMPARATIVE POTENCY TESTS ON GERMAN AND AMERICAN TETANUS ANTITOXIN UNITS

No. of pigs	Average wt. gms.	Toxin	Dose c.c.	Antitoxin	Dose	Survived days-hours (average)
4	360	American G	0.00077	American T 28	1/10 i. u.	4 17
4	368	»	0.00080	»	1/10 i. u.	3 11
4	362	»	0.00083	»	1/10 i. u.	2 22
2	345	»	0.00075	German no number	1/666 AE	3 10
2	377	»	0.00080	»	1/666 AE	3 7
2	345	»	0.00085	»	1/666 AE	2 17
2	382	»	0.00090	»	1/666 AE	2 23
2	347	»	0.00095	»	1/666 AE	2 1
2	385	»	0.001	»	1/666 AE	2 2

II — ANTI-MENINGOCOCCUS SERUM

MENINGOCOCCUS TYPES IN DENMARK

REPORT

By Th. Madsen

Based on investigations by F. Wulff and E. Vollmond.

1919-1920. — 283 strains were examined; the types were as follows :

Strains from	Number	Type
a. Blood from petecchial meningococcus sepsis.	15	A
b. Spinal fluid.	46	A
	1	B
	1	C
	1	D
	1	E
c. Naso-pharynx.	93 (15 from patients with meningitis)	A
	61	F
	66	different types.

The division of types was used provisionally until comparison could be made with the English types. The comparison with Gordon's types showed :

Gordon's type		Danish type	
I	=	F	(non-pathogenic pharynx strain) never found in patients.
II	=	A	a strain only once found in the pharynx of a healthy person (see later).
III	=	D	(only once found in the spinal fluid of a meningitis patient).
IV			not found.

1921. — Cultures examined from 11 spinal strains { 10 type A. / 1 not type A.

In 419 healthy soldiers 41 meningococcus carriers were found with 6 of type A.

1922. — Cultures examined from 6 spinal strains { 4 type A. / 2 not type A.

In 492 healthy soldiers 121 carriers were found with 27 of type A.

From this it is obvious that type A in the majority of cases is the pathogenic type in Denmark. It was therefore of great interest to compare it with the English types. Dr. Gordon very kindly undertook this examination and defined it as an *atypical type II strain.* Our investigations give the same result, because

(1) Type A is only agglutinated by type II serum;

(2) Type A almost entirely absorbs the majority of type serum II; the close relation to type II is therefore obvious. On the other hand, these two are not quite identical because the Gordon type II does not absorb our Danish type serum A.

It seems that the Danish type A is more clearly differentiated as regards its agglutinogen composition than the English type II, the English type serum II being entirely absorbed by the Danish A type, but not *vice versa.*

None of the strains from England or those mentioned in the following table have absorbed type serum A.

Danish type A must therefore be considered closely related to, but not quite identical with, type II. It has not hitherto been found outside Denmark.

In 1922 we had the opportunity of examining the following foreign strains :

	Total	Group I	Group II	Group (Basel Rostock)	?
Germany (Rostock)	5		3	2	
» (Bremen)	2		2		
» (Berlin)	10	7	3		
Poland (Warsaw)	7	4	3		
Switzerland (Basel)	2			2	
Sweden (Stockholm)	2		2		
Palestine (Jerusalem)	1 (*)		1		
Turkey (Constantinople)	2 (*)				2

This definition has been made by means of the types kindly sent us from "The National Collection of Type Cultures" in England. In accordance with Griffith and Scott, the 4 Gordon types form two Groups, group 1 (Gordon's type I and III) and group II (Gordon's type II and IV). Most of these strains could be referred to group I or II, but this was not the case with 2 strains from Rostock and 2 from Basel. The production of specific serum against these 4 strains seemed to show that they belonged to a special group, provisionally called Basel-Rostock group. Two carbolic emulsions from Constantinople could not be agglutinated by our sera; the lack of sufficient material prevented further definition.

The low prevalence of epidemic cerebrospinal meningitis during the last year has prevented more intensive investigations on this line. These investigations should be continued.

An international nomenclature for the meningococcus types should be aimed at.

(*) Carbolic emulsion.

MENINGOCOCCUS SERUM

REPORT

By Th. Madsen

Based on experiments by J. R. Mörch.

As the therapeutic principle of the meningococcus serum is still unknown, different antibodies have been used for its standardisation in the hope that a high concentration of these might also be an indicator of a high concentration of antibodies of therapeutic value. Under these conditions, it is obvious that it would be advantageous to have a serum containing the greatest possible number of these antibodies, which we are at present able to measure.

As we still lack information on the details of production of the different antibodies in immunised animals, experiments of this kind have been carried out on an extensive scale.

The serum was obtained from horses treated with :

(1) Emulsion of living meningococci from ascitic agar cultures, injected intravenously in increasing doses, last dose before the bleeding being about 10 cc., each containing 50,000 millions of microbes.

The antibodies examined were *agglutinins, complement-fixing antibodies* and *bacteriotropins.*

(2) *Agglutinins.* A carbolic emulsion of an 18h. culture on ascitic agar (Ph. 7. 30) heated to 60° 1/2h. containing 450 millions pr. cc. was used. Reading after 18h. at 56°; titer = total agglutination.

Complement-fixing antibodies. — The antigen was an aqueous extract of meningococci.

Bacteriotropins determined after *Neufeld.* — The reading performed with a phagocytosis corresponding to 20-30 microbes per leukocyte.

The curve shows the results, which entirely correspond with those obtained in a series of other similar experiments (curves 1 and 2, see pages 36 and 37).

The curves show the important fact that the three antibodies do not run parallel.

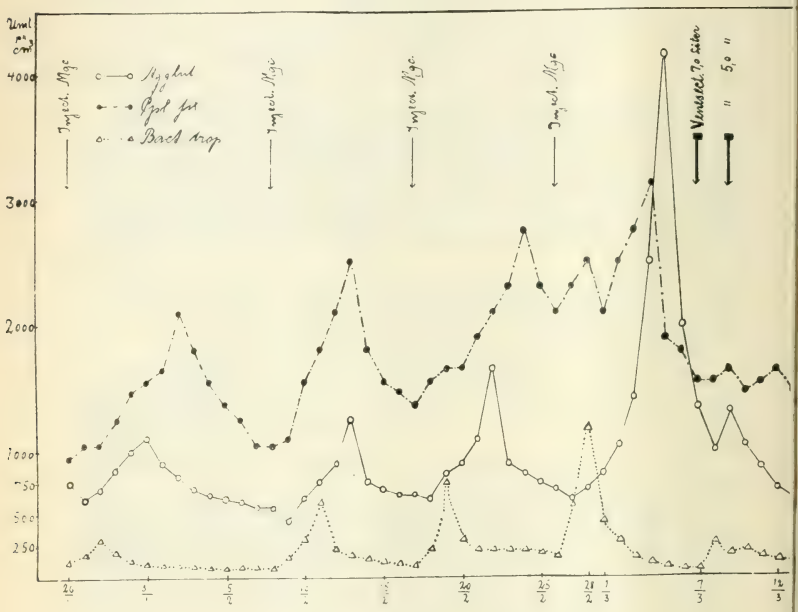
The *agglutinin-curve*, after a negative phase only slightly marked, rises gradually to reach its climax 5-7 days (as a rule 5 days) after the last injection of antigen.

The *complement-fixing bodies* have no negative phase, but rise gradually after the injection to reach their climax in 5-7 days.

The two antibodies show a great concordance as regards their

formation curve, but they do not run parallel, and it is obvious that their climax often does not occur simultaneously. At this point of the curve a period of 24 hours is sufficient completely to change the mutual relation between them.

The *bacteriotropins* rise rapidly after the injection and reach a climax as soon as 2-3 days (generally 2) afterwards, followed by a steep fall. *This curve is therefore quite different from the two former ones.*

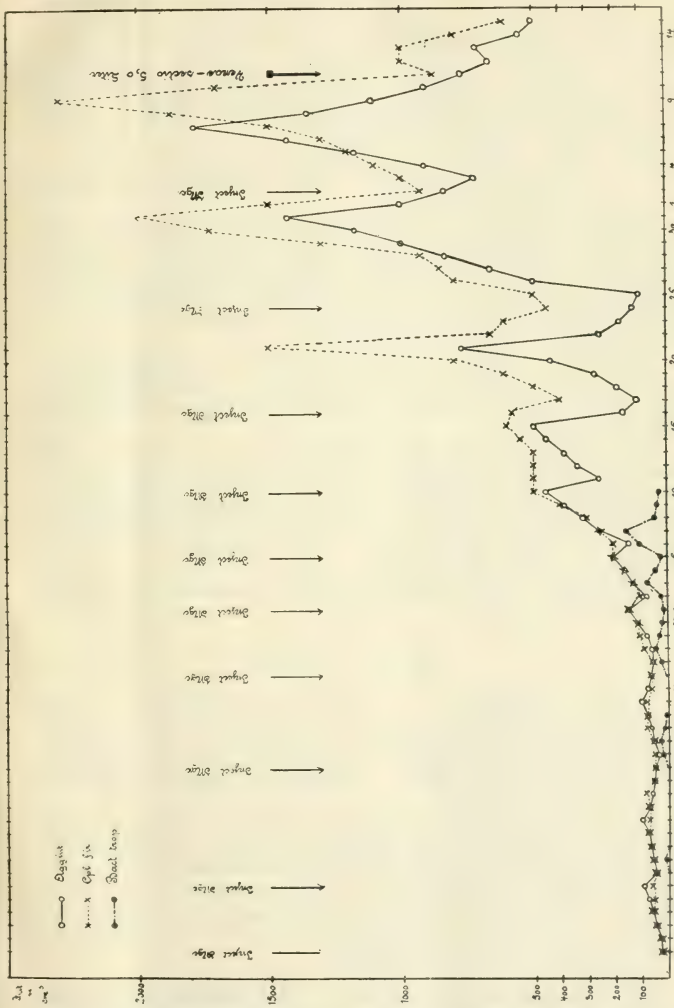


No. 1

These facts, which are confirmed by a series of quite concordant experiments, are sufficient to show that the presence of a large quantity of one of these antibodies does not guarantee the existence of a corresponding quantity of the other antibodies.

These curves warn us that the date usually chosen for the bleeding of the serum-producing horse is not the most suitable. The usual method is to bleed about 9 days after the injection of antigen. The curves show that, if the object is to obtain a maximum of agglutinin or complement-fixing antibodies, the 5th day would be the best. But if the object is to obtain a large quantity of bacteriotropins, the bleeding should be advanced to the second day.

The difference between these antibodies is emphasised by the fact that they are attached to different protein-fractions of the



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immunserum. By precipitation with $(NH_4)_2\ SO_4$ M. Mörch has demonstrated that the *Agglutinin* follows the *euglobulin* fraction, whilst the *bacteriotropins* are found in the *pseudo-*

globulin fraction; on the other hand, it has been found impossible to isolate the *Complement-fixing antibodies :* they appear in all the fractions in quantities, almost corresponding to those of the other two antibodies : resp. agglutinins and bacteriotropins.

It seems, moreover, that the complement-fixing bodies connected with the euglobulin and those connected with its pseudoglobulin present a different *thermoresistance*, the former being relatively thermostable, the latter being destroyed together with the bacteriotropins after exposure 1 1/2h. at 56°.

This might indicate that the complement-fixing substances cannot be separated from the two other antibodies, and might even be identical with them.

On the other hand, the curves seem to indicate that also other bodies in the meningococcus serum help to account for its complement-fixing power; therefore this characteristic might be taken to indicate the action of *all* the antibodies in question and at present it may be considered the most suitable basis for titration, until the specific body has been found.

All the mentioned qualitative experiments have been carried out with a homologue strain (Danish type A). For standardisation it is, of course, important to know the results of a comparison with another type.

Curve 3 (see page 39) shows that when a serum prepared with type A is measured simultaneously with this strain and another meningococcus *type,* the form of the curve is identical for all three antibodies, but the *strength* of the serum is much greater (in this case about double) in the case of a homologue strain than in the case of a heterologue strain.

This fact is clearly important in the case of an international standardisation.

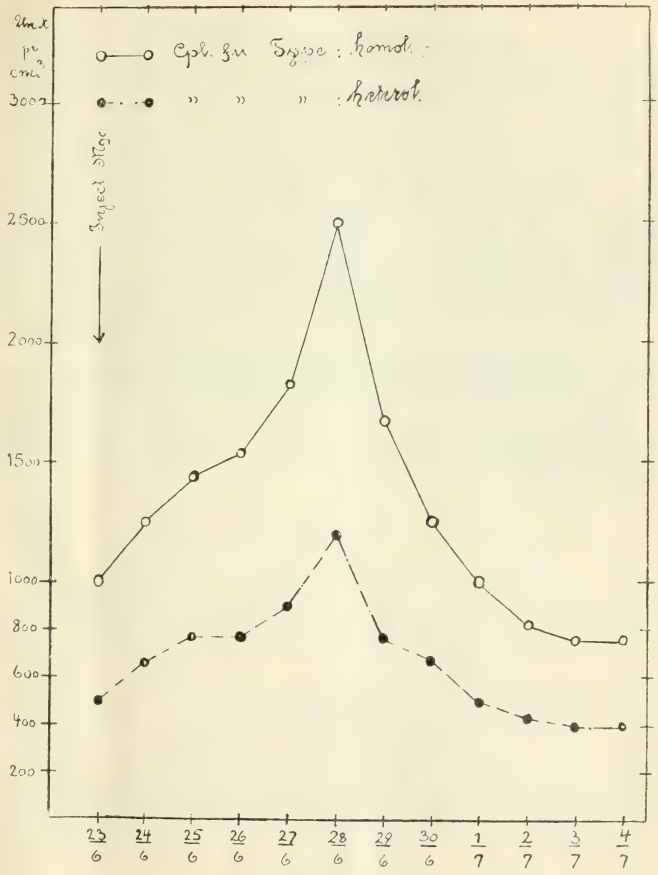
TECHNICAL REMARKS

1. *The agglutinin test.* — The quantitative results depend entirely upon the strain used for the emulsion, the substratum upon which the meningococci are grown, the time and temperature allowed for the culture. Comparative titrations therefore present many difficulties.

Much better results are obtained by using as antigen an *alcoholic extract of meningococci,* diluted with 0.9% NaCl. This extract, together with meningococcus serum, gives a good precipitation, easy to measure quantitatively. This reaction seems to be specific for meningococci, even to a certain degree type specific: this antigen is quite stable and seems to be much more suitable for constant titration of the agglutinins.

2. *The complement-fixation test.* — It is difficult to obtain a constant quantitative titration, even with dried microbes; a better antigen might perhaps be obtained by the use of an alcoholic extract of microbes, but even with a constant antigen the well-known variations in the comparative titration could scarcely be

avoided (see my report on the serodiagnostic of syphilis). An international standardisation based on this test will be difficult to obtain.



No. 3

3. *The bacteriotropins test* seems to be more stable, but the technique is difficult and requires great practice and care. The evaluation differs according to the different strains of meningococci, even when of the same type.

Experiments with the protective power of antimeningococcus serum have not yet been undertaken.

The time has not yet arrived for setting up an international standard for the meningococcus serum. The most general opinion seems to be that the complement-fixation test, as a kind of common denominator for the different antibodies, would be the method most likely to express its strength. But as we do not yet possess a method ensuring constant comparable results, another line might be explored. Although the agglutinins and the complement-fixing antibodies are not identical, the curves show a remarkable correspondence, so that provisionally the agglutinin test may give satisfactory results. The advantage of this test is that a constant antigen can be produced (alcoholic extract of the microbes). By this test and some standard sera, a provisional international standard might be possible. Certain questions must first be settled :

(*a*) The quantitative proportions of different antibodies in a large number of sera must be determined;

(*b*) The thermolability of the agglutinins must be investigated (the results obtained by us differ from those obtained by others);

(*c*) Titrations must be performed with different antigens and with different sera.

RESULTS OF EXPERIMENTS ON THE VARIOUS TYPES OF MENINGOCOCCI AND THE TITRATION OF ANTI-MENINGOCOCCUS SERUM

Report by Professor Dopter,
Pasteur Institute, Paris.

1. *Comparative study of the various types of meningococci.*

For a complete study, a comparison would have to be made between the samples of cultures from different laboratories. Owing to the well-known fragility of these cultures if left for several days at a normal temperature, the samples which reached the Pasteur Institute did not survive the journey and the prolonged delay at the Customs.

The tests could only be carried out on the samples which were cultivated at the Pasteur Institute, and on those which were brought from London and delivered by Dr. Gordon.

Gordon's types 1, 2, 3 and 4 were compared with types A, B, C and D of the Pasteur Institute by means of agglutination tests, both by the classical slow process and by the saturated serum process. Anti-sera A, B, C and D, and the sera obtained by inoculating horses with each of the Gordon samples, were used for these tests.

This comparative study gave the following results :

(1) Gordon's types 1 and 3 should be combined into one type, corresponding to type A of the Pasteur Institute. The latter could not be determined separately, as the agglutinations turned out to be all equal, whether they were carried out with anti-serum I or anti-serum III.

(2) Gordon's types 2 and 4 correspond to type B of the Pasteur Institute. The observation made above, regarding the impossibility of dissociation, applied also to type B.

(3) In addition to these varieties, there are two types which are specifically different from those already mentioned : types C and D of the Pasteur Institute. The latter, however, is exceptional, and has only been met with once.

2. *Research regarding a titration process.*

The titration of anti-meningococcus serum by determining its agglutinative and sensitising power is impossible, as the results vary according to the strain of meningococci employed. Moreover, from the fact that a serum is rich in amboceptors, it unfortunately does not follow that it possesses a high curative value; these methods cannot provide any accurate indication of its therapeutic

value. They give some idea of the intensity of the reactions in the organism of the animal inoculated, but nothing more.

Further, the Pasteur Institute sera, if intended for therapeutical purposes, are heated; heating makes the agglutinins disappear; but, despite their absence, experience shows beyond doubt that the anti-meningococcus serum is endowed with a curative power where human infection is concerned.

Titration by measurement of the anti-endotoxic power of the serum has been attempted on rabbits, mice, and young guinea-pigs.

Rabbits cannot be used, as the differences in individual resistance between animals of the same age and weight make any comparison useless.

Mice are not sufficiently susceptible to the action of the toxin, even intravenously injected, to be of any use.

It is only guinea-pigs weighing from 80 to 100 grammes that can be used for these tests. The experiments which were carried out confirmed the previous data, namely, that a cubic centimetre of an active serum can neutralise from 5 to 10 lethal doses. However, the irregularity of the results obtained with the same serum and the same toxin and the small margin observed in the determination of the anti-endotoxic power, tend to limit considerably the value of this method; moreover, the method appears to be of very slight interest, for in the pathogenic effects of the meningococcus on the human species, the infectious element clearly predominates over the toxic element.

We shall now consider the determination of the bactericidal power.

In most cases it is impossible to determine the value of this power owing to the considerable differences of individual receptivity between animals of any species when injected with an equal dose of the culture. Attempts were made to overcome these difficulties by passing the meningococci several times through guinea-pigs and mice, and by the use of bags of collodion; but the results have been negative.

The so-called peritoneal test, which produces a kind of *Pfeiffer* reaction, is the only one which allows of the desired estimation, for the peritoneum of every animal under experiment, as it receives only doses which are almost always fatal, shows an identical defensive reaction. It is thus easy to establish a comparison.

The experiment consists in injecting into the peritoneum dilutions, at various strengths, of unheated anti-meningococcus serum and then, 24 hours later, a 1/6th culture of meningococci on gelatine; the peritoneal exudate is tapped at varying intervals by means of a pipette, and the gradual lysis and complete disappearance of the meningococcus may be observed.

By the use of decreasing quantities of anti-serum on a batch of guinea-pigs, we may succeed in determining the dose which can make the meningococcus disappear in, for example, 30 minutes. A serum which, given in a dose of 0.25 cubic centimetres, produces the disappearance of the germs within this period may be used in human therapeutics.

The results obtained are constant; but, as in the case of the test of anti-endotoxic power, the margin left for the estimation of the anti-infectious power is not sufficiently wide, and the process cannot be used for purposes of titration.

In point of fact, no precise method of dosing has yet been found; the goal will only be attained when it is possible to determine the minimum lethal dose of meningococci which can kill the animal within a constant period and the dose of serum required to counteract it.

INVESTIGATIONS WITH REGARD TO MENINGOCOCCI

By F. Neufeld,

Robert Koch Institute, Berlin.

I. We have so far been unable to obtain a sufficient number of strains of meningococci to form an opinion regarding the frequency of the various types in Germany. Type A, which is frequent in Copenhagen, has as yet only once been discovered here. Subsequent investigations shall extend, as far as possible, to different parts of Germany.

II. As far as the methods of titrating the sera are concerned, I am also not yet in a position to make any definite proposal. The present state of affairs is undoubtedly unsatisfactory. In the official tests in Germany (phagocytosis and complement fixation), no attention is paid to the various types. In America, the method of standardisation by the phagocytosis test has been abandoned and replaced by the agglutinin test. In France, the complement-fixing antibodies are tested. But we know that both antibodies are not protective substances. Such tests should therefore be regarded as nothing more than makeshifts. If the sera were always prepared in the same manner, it is indeed possible that the agglutinins, for example, might nevertheless serve as a useful index of the protective power of a serum, since their quantity might correspond more or less to that of the protective antibodies. On the other hand, sera which have been prepared in a factory by different methods frequently will fail to show this relation.

In England, Gordon especially, as a result of careful investigations, has advocated the adoption of anti-endotoxins as a standard of measurement, but so far the accurate quantitative measurement of these substances does not appear to be possible. In my opinion, further investigations as regards meningococcus opsonins and anti-endotoxins are necessary. In these investigations, the practice of the English scientists should be followed, and the sera tested correspondingly in the laboratory and in their action upon patients. Should it really be proved that the anti-endotoxins alone—and not the opsonins—correspond to the curative power of a serum, then the test proposed by Gordon ought, in my opinion, to be generally adopted. Those sera in which the contents of anti-endotoxins and opsonins differ widely should be submitted to different laboratories for investigation. As regards examination by means of the phagocytosis test, it is worthy of note that, according to the report of the Medical Research Council (Special Reports No. 50), the technical difficulties of this test have not been entirely

overcome by the authors and that their results therefore contain an element of doubt in this respect.

III. It appears to me desirable that the observations communicated by Dr. Glover in the above-mentioned English report on the percentage of healthy carriers of meningococci under different conditions should be made the subject of further examination.

THE STANDARDISATION OF ANTI-PNEUMOCOCCUS AND ANTI-MENINGOCOCCUS SERA IN THE UNITED STATES OF AMERICA[1]

By Augustus Wadsworth

There are two standards in the United States: the Federal standard and that of New York State. Previous to Federal standardisation, the commercial sera lacked potency and New York State established the first standard. A federal standardisation followed immediately and it has been developed step by step from the simple testing and approval of potent sera by the Hygienic Laboratory in Washington to the distribution of standard culture and sera for comparative tests. There is still no official standard; but instead all sera are tested and passed by the Hygienic Laboratory before they are sold. New York State adopted the standards and methods of the Rockefeller Institute. The standards and methods for anti-pneumococcus serum have stood without change. The methods of preparing anti-meningococcus serum have been improved and the standards materially raised. In the standardisation of New York State, only the essentials have been defined; methods in question that are not essential have been left optional.

Basic principles of the standard of New York State.

The serum must be labelled as to potency.

The serum must be tested by prescribed methods.

The serum must equal or exceed a minimum standard.

Basic principles of the Federal standard.

The serum must be tested by prescribed methods.

The results of the tests, together with samples of sera, must be submitted to the Hygienic Laboratory in Washington. They are there tested and passed before they can be sold in inter-State commerce.

Labelling as to potency is not required and it is not practised because there is no official standard.

Official requirements

Copies of the requirements of New York State accompany this statement together with the minimum requirements of the Hygienic Laboratory in Washington as issued on April 8th, 1921.

Methods of testing these sera[1]

Anti-Pneumococcus Serum.

The Federal and State methods of testing the protective action of the serum are similar, differing in minor technical procedures.

The standards of virulence are similar and the maintenance of virulence differs only in minor details. The serum and culture are mixed in a syringe and injected two minutes later in the New York State method and in the method of the Rockefeller Hospital. The upper limit of potency is also determined by testing increasing quantities of serum. In the Federal test the serum is injected and then the culture a few minutes later. Only one quantity of serum, the minimum required, is tested; a larger number of controls is therefore necessary in the Federal test.

Anti-Meningococcus Serum.

In New York State the official standard is the agglutinative titre. No other test is required. There is no official Federal standard, but the agglutination, complement-fixing and bacteriotropic activities are tested. The federal method of performing the agglutination test differs from that of New York State and the Rockefeller Institute in many details, chiefly, however, in using heated antigens instead of living cultures and in reading the test.

The Federal regulations require immunisation of the horses with twelve strains of the meningococcus which are distributed to the manufacturers. New York State requires only the four strains established as standard cultures and actually uses in the preparation of all its sera only six strains. The details of the establishment of the standards, the selection of standard cultures representative of the different types of the meningococcus and the methods of immunisation are published in the following articles : Standardisation of Anti-Pneumococcus and Anti-Meningococcus Sera, *Archives of International Medicine,* 1919. Vol. XXIII, pp. 269-281; Study of the Classification of Meningococci, *Journal of Experimental Medicine,* January 1921, Vol. XXXIII, No. 1, pp. 99-105; Comparison of the Potency of Polyvalent Anti-Meningococcus Serum produced with four and six representative strains and that produced by sixty strains as determined by the Agglutinative Titre, pp. 107-118.

Subsequent observations of the therapeutic action of these sera and subsequent study of the agglutinative action of the sera, prepared by immunisation with one, four, six, twenty and sixty strains, confirm the conclusions already published that the maximum reaction is obtained with six strains, two from each of the three recognised types of meningococcus found in America.

Therapeutic potency of different standards

Satisfactory data concerning the relative therapeutic value of different products is difficult to obtain. The experience of the Rockefeller Institute is the most complete, the most varied and covers the largest series of cases of any observations made in America. They conclude that the therapeutic action in general corresponds to the agglutinative titre. From time to time isolated cases which do not yield to treatment have been encountered.

Cultures from these cases which failed to agglutinate with the serum were added to the strains which were used to immunise the horses. The methods of the Rockefeller Institute were adopted by the Laboratory of the State of New York and the sera, so far as it has been possible to determine, have corresponded in action closely. There is nothing to indicate that the new sera of the last three years produced with only six strains have less therapeutic value, rather the contrary.

Practical problems for future investigation

The data concerning the therapeutic action of different anti-meningococcus sera should be more definite. The culture of the meningococcus obtained from cases in which the serum has not been effective should be carefully studied in all their immunological reactions as soon as possible after isolation before cultural attenuation has taken place. These studies should be made with the serum that has not been effective and with other sera as well. Sera which vary in their action should also be tested with a number of strains representative of all types of the pathogenic meningococci. For the purposes of these studies, there should be an interchange of cultures and sera between all the laboratories interested in this work. In the absence of satisfactory laboratory methods of determining directly the therapeutic potency, study along these lines will be of the greatest practical value.

A careful study of the therapeutic action of type 1 anti-pneumococcus serum in the early treatment of type 1 cases of pneumonia should be made in one or more representative institutions fully equipped for such an investigation. Close co-operation between the laboratory and the hospital is essential to a detailed study of the treatment in each case.

Conclusions regarding standardisation

Experience with the standardisation of both anti-pneumococcus and anti-meningococcus serum in the United States during the past five years as contrasted with the previous lack of any standardisation demonstrates its practical value despite the fact that the methods are not in all respects satisfactory and in a measure arbitrarily established. Formerly, the reports of the failure of these two sera in the treatment of cases were quite numerous; now they are less frequent. This statement applies more particularly to anti-meningococcus serum because anti-pneumococcus serum has not been so generally used.

In the light of this experience in the United States, the essentials of an effective standardisation may be formulated as follows :

(1) An official standard of potency should be set.

(2) The methods of testing should be outlined in principle only, leaving optional the technical procedures about which there is a difference of opinion.

(3) Standard cultures representative of the different types of pathogenic meningococci should be selected and distributed.

(4) Standard or control sera representing the minimum potency acceptable should be prepared and distributed.

(5) The potency of the sera as tested should be recorded in order that the precise character of the serum may be generally known.

(6) The minimum requirements of the methods of immunisation to be used by manufacturers should be prescribed. The standard cultures which are used in the potency test should be used in the production of the serum. The minimum periods of immunisation should be prescribed.

(7) If the serum is polyvalent in its action, the potency test should be a measure of polyvalency.

In conclusion, it would seem wise for the committees of the Conference to proceed first to the consideration and adoption of certain fundamental principles which appear to be essential to a practical standardisation and then to discuss the practical value of the different methods in fulfilling these requirements.

Annex I.

MINIMUM REQUIREMENTS

FOR ANTI-MENINGOCOCCIC, ANTI-PNEUMOCOCCIC

Duplicate samples of at least 15 cc. are to be sent, each lot as ready for the final container, after all treatments such as the mixing in of preservative and filtration are completed and after the lot has passed satisfactory tests at the establishment.

Protocols of these tests shall be sent to the Hygienic Laboratory.

The sera should be free from excessive colour or turbidity. The maximum intensity of colour should be that represented by 0.3 per cent. saturated solution of bismarck brown in absolute alcohol, and the maximum turbidity that of 200 parts per million of silica. There should be no gross particles in the serum.

Anti-meningococcic sera are considered satisfactory which show an agglutination titre or a complement fixation titre against each antigen at least equal to that shown by the control serum furnished by the Hygienic Laboratory.

Anti-pneumococcic sera are considered satisfactory which show protection of at least as great a percentage of mice as are protected by the control serum furnished by the Hygienic Laboratory. At least two tests of three mice each shall be made of each serum, not less than 24 hours apart, and the resultant protection shall be compared with the protection of the two sets of

mice receiving the control serum. No set of tests shall be considered except those which show some mortality, but not complete, with the control serum.

Control anti-meningococcic, and anti-pneumococcic sera will be furnished from time to time to licensed establishments on application.

Annex II.

THE SANITARY CODE ESTABLISHED BY THE PUBLIC HEALTH COUNCIL OF THE STATE OF NEW YORK

Chapter IX

(Adopted December 27th, 1917.)

Regulation 1. Sale of anti-pneumococcus and anti-meningococcus serum regulated.

No serum for the treatment of pneumonia or of meningitis shall be sold or offered for sale in the State of New York unless each package is accompanied by a label or circular on which is stated the potency of the serum as tested by the methods established by the rules and regulations of the State Commissioner of Health : and no such serum shall be sold or offered for sale the potency of which does not equal or exceed the minimum fixed in such rules or regulations.

This regulation shall take effect on February 15th, 1918.

RULES AND REGULATIONS FOR THE TESTING OF THE POTENCY OF ANTI-MENINGOCOCCUS SERUM

(Effective November 1st, 1922.)

I. *Type of Serum.*

Anti-meningococcus serum for the treatment of meningitis which is offered for sale in the State of New York shall be prepared by the immunisation of animals for at least three months against at least four standard cultures representative of the principal types of the meningococcus.

II. *Minimum Standard of Potency.*

The potency of anti-meningococcus serum intended for therapeutic use, when tested and compared with the standard serum

supplied on request by the Laboratory of the New York State Department of Health, shall have an agglutinative action in dilution : 1-5000, 1-2000, 1-3000 and 1-1500 with the four standard cultures of the meningococcus A, B, C and D respectively, under conditions in which a standard suspension is employed and the tubes maintained for 16 to 24 hours at 55° C.

III. *Standard Cultures.*

The standard cultures of meningococcus representative of the principal types may be obtained from the Laboratory of the State Department of Health. They are cultures which have been identified by serological tests and which possess distinctive agglutinative and antigenic properties.

IV. *Standard Suspensions.*

The standard suspensions for performing the agglutinating tests are so prepared as to correspond in opacity with a standard suspension of barium sulphate containing 3 cc. of a 1 per cent. barium chloride solution in 97 cc. of a 1 per cent. sulphuric acid solution. Such a standard meningococcus suspension contains approximately 2,000 million cocci per cc.

V. *Method of Testing the Potency of the Serum.*

Normal saline solution (0.85 per cent.) should be used as the diluent. After dilution with the serum the final mixture should contain 1,000 million cocci. The standard culture suspension should be added in agglutination tubes (8 to 11 mm. in diameter) to the diluted sera to be tested, and also to similar dilutions of the standard sera. These test mixtures should be incubated at 55° C. for from 16 to 24 hours. The titre thus determined is the maximum dilution of the serum in which definite clumping of the meningococcus in the suspension can be easily detected by the unaided eye. The agglutinative titre which is to be recorded is the potency of the serum.

VI. *Physical Properties.*

The serum shall be free from all the cellular or solid elements of the blood and should not contain traces of hemoglobin or bile which can be detected by the unaided eye. In order to facilitate inspection, the serum should be put up in containers of colourless transparent glass.

VII. *Sterility Tests.*

The sterility tests of the final product shall comply with the requirements of the United States Public Health Service.

VIII. *Expiration Date.*

Expiration date for the sale of the serum shall comply with the requirements of the United States Public Health Service.

RULES AND REGULATIONS FOR THE TESTING OF THE POTENCY OF ANTI-PNEUMOCOCCUS SERUM

I. *Type of Serum.*

Anti-pneumococcus serum for the treatment of pneumonia which is offered for sale in the State of New York shall be prepared by the immunisation of animals against pneumococcus of Type 1.

II. *Minimum Standard of Potency.*

The potency of the anti-pneumococcus serum intended for therapeutic use shall equal that of the standard serum prepared by the laboratory of the State Department of Health and which is supplied on request. The standard serum is of such potency that, under the conditions prescribed, 0.2 cc. will protect a mouse 16 to 22 grams in weight for at least 4 days against at least 0.1 cc. of the standard culture of pneumococcus Type 1, 0.000001 cc. of which will kill the control mouse in less than 48 hours.

III. *Standard cultures.*

The standard culture of pneumococcus Type 1 employed for testing purposes may be obtained from the laboratory of the State Department of Health. However, before the protection tests are performed, the virulence of the culture shall be determined by experiment to be such that 0.000001 cc. of a 16-to 18-hour broth culture will kill a mouse of 18 to 22 grams weight within 48 hours.

IV. *Method of Testing the Potency of the Serum.*

The dilution of culture and of the serum shall be made in broth and prepared immediately before the test is performed and in such manner that no less than 0.5 cc. are measured. Thus 2 cc. of the serum are diluted with 3 cc. of the broth, so that 0.5 cc. of the mixture will contain 0.2 cc. of the serum. Similarly 1 cc. of the culture is added to 4 cc. of the broth, so that 0.5 of the mixture will contain 0.1 cc. of the culture.

In making the injection, 0.5 cc. of the diluted culture and 0.5 cc. of the diluted serum are thoroughly mixed in the barrel of the syringe and in two minutes injected into the peritoneum of a mouse weighing from 16 to 22 grams. Moreover, control tests with

standard serum and control tests of the virulence of the culture without the serum are made at the same time. The protective titre of the serum against the culture is the potency and should be recorded as equal to or in excess of the standard serum.

V. *Physical Properties.*

The serum shall be free from all the cellular or solid elements of the blood. In order to facilitate inspection, the serum should be put up in containers of colourless transparent glass.

VI. *Sterility of the Serum.*

Sterility tests of the final product shall comply with the requirements of the United States Public Health Service.

VII. *Expiration Date.*

Expiration date for the sale of the serum shall comply with the requirements of the United States Public Health Service.

III — ANTI-PNEUMOCOCCUS SERUM

THE OCCURRENCE OF DIFFERENT TYPES OF PNEUMOCOCCI IN DENMARK

By Th. Madsen

Based on investigations by Dr. S. Christensen on 233 patients and 1,474 healthy persons.

1. *Patients.* — 110 cases of *pneumonia crouposa :*

Type I (American types) found in 36 cases (33 %) with 8 deaths : case mortality rate 22 %.
— II (American types) found in 30 cases (27 %) with 2 deaths : case mortality rate 7 %.
— III (American types) found in 9 cases (8 %) with 2 deaths : case mortality rate 22 %.
— IV (American types) found in 35 cases (32 %) with 1 death : case mortality rate 3 %.

2. — 16 cases of *meningitis pneumococcica :*

Type I in 5 cases
— III in 5 — } all fatal.
— IV in 6 —

3. *Influenza.* — In 31 % of the patients pneumococci were found :

Type I in 4 cases (6 %).
— II in 3 — (4 %).
— III in 10 — (14 %).
— IV in 52 — (75 %).
Total. . . 69 cases.

4. *In 1,474 healthy persons.* — Pneumococci were found in 230 healthy persons out of 1,474 examined :

Type I in 1 case.
— II in 3 cases.
— III in 21 —
— IV in 205 —
Total. . . 230 cases.

Thus the same relative grouping of the types was found in Denmark as in the U. S. A. and England.

In the case of 26 persons, several colonies from the original plate were examined with regard to type :

In no case was more than one type found.

In no case did we succeed in changing the type of pneumococcus by continuous cultivation *in vitro* or *in vivo*. These experiments were not sufficiently numerous.

It was stated that for 200 different strains of pneumococci the same grouping of types was obtained by the *agglutinin-absorption* test as by the simple *agglutination* test.

A comparison between the *agglutination test* and the *complement-fixation test* was carried out. When unwashed pneumococci were used, the results were indefinite, both with the simple complement-fixation test and with the complement-fixation after absorption. Sera often became auto-inhibitory after treatment with the culture of pneumococci the absorption power of which was to be examined. It was stated that this difficulty could be removed by *washing* the pneumococci twice before they were used for the absorption or complement-fixation test.

(For details see : S. Christensen, *Comptes rend. Soc. Biol.* 1922. p. 459.)

With this improved technique, 150 experiments with complement-fixation after absorption were performed, and the same type-grouping as with the agglutination test was constantly found.

ANTI-PNEUMOCOCCUS SERUM

By I. Cotoni,

Pasteur Institute, Paris.

The investigations which we have carried out since the last Conference confirm our view that neither the *agglutinating power* nor the *fixing power* can be relied on at present for the measurement of the therapeutic power of the anti-pneumococcus serum.

The most appropriate method is still the titration of the *bactericidal power* in mice. It is, however, only employed for lack of some better method. In fact, it is proved by long experience that the susceptibility of mice to equivalent doses of pneumococcus cultures varies very considerably; endeavours are therefore made to reduce the possibility of error, as far as possible, by using a large number of mice, but the difficulties are greatly increased when the sera to be titrated are very numerous. It is therefore to be hoped that the discovery of a new method of titration *in vitro* may enable us to eliminate the variable factor of individual resistance.

We are still opposed to the method of intra-peritoneal injection as being too random a method with small animals. The question arises as to which is the better method of the two : the one of preventive, or of the simultaneous, injection. We nearly always employ the former, giving subcutaneously an injection on the first day of 1/10th cc. of the serum, using mice of 15 to 20 gr., and followed by an injection on the following day of 10, 100, 1,000, 10,000, etc. lethal doses of the culture. However, we have obtained good results also with the method of simultaneous injection and we are at present endeavouring to render it more simple and practical.

As regards the question of monovalence and polyvalence of sera we still consider, as we explained in " Pneumococci and Pneumococcus affections : Monograph of the Pasteur Institute, 1922," that a serum prepared by the use of a strain of pneumococcus may exert considerable preventive power in mice infected with pneumococci, belonging, as regards their agglutinating properties, to a different group. One of the many examples which illustrate this fact is that a dose of 1/10th cc. of a certain serum, prepared with a pneumococcus of type III, protects mice against inoculation on the following day with 1,000 lethal doses of a pneumococcus type II. Sera I and II would provide similar instances. However, it is necessary to add that polyvalence varies to a considerable extent according to specimens of sera under examination, and cannot be regarded as a constant property of anti-pneumococcus sera. This observation confirms the necessity of a careful titration of the serum.

October 18th, 1922.

PNEUMOCOCCI AND PNEUMOCOCCUS SERUM

By Fred. Griffith, London.

Types of Pneumococci obtained from Cases of Lobar Pneumonia

Summary.

This Report (Ministry of Health, No. 13, 1922) deals with an enquiry into the incidence of the various types of pneumococci in cases of lobar pneumonia derived mainly from the London area, together with some observations on the technique in the diagnosis of type and on the question of the immunological independence of types.

Distribution of Types (1) *in the Present Series of 150 Cases.*

Type	I	II	III	IV
No. of cases	46	49	10	45
Percentage of total cases	30.6	32.7	6.7	30

The above results are in fairly close agreement with the American results; they found in 454 cases of lobar pneumonia Type I 33.3 per cent; Type II 29.3 per cent; Type III 13 per cent.; Type IV 20.3 per cent.; and Atypical Type II 4.2 per cent. In the above table, strains corresponding with the American Atypical Type II are included under IV.

Atypical Pneumococci.

Such strains were at first classed by the American observers as Type IV, and were thought to be serologically unrelated. Later observations have shown that they might be subdivided into an indefinitely large number of serological types.

As will be seen from the above table, 45 strains of pneumococci from cases of lobar pneumonia failed to agglutinate with any of the type sera I, II or III. An attempt has been made to classify these strains into types, together with 32 strains from cases of other diseases, principally of respiratory origin. Agglutinating sera were prepared from 12 of the strains, and were tested against each of the 77. Out of the 77 strains 49 were agglutinated in a specific manner; the remainder did not agglutinate with any of the 12 sera.

Serological Analysis of 49 strains of Group IV with 12 Agglutinating Sera of different Types.

Type sera	IIA	IIB	C	D	E	F	G	H	I	J	K	L	= 12
Total cases	8.	12.	5.	5.	3.	3.	2.	2.	2.	2.	1.	4.	= 49

(1) Under IV are included all strains not reacting with Types I, II and III sera.

Varieties of types yielded by the same patient. — In a considerable number of cases of pneumonia, two or more serological types of pneumococci have been obtained from the same sample of sputum. These results emphasise the need for caution in deciding on the type of infecting pneumococcus, at least where the material is not obtained direct from the diseased lung.

General Conclusions.

A. Pneumococci should be classified as far as possible according to their serological types, and for this purpose the technique adopted should be uniform in all essential respects.

(1) In view of the occurrence of more than one type in the same sputum, there is a greater degree of probability that the infecting type would be obtained if the material was first inoculated into a mouse.

(2) Strains of pneumococci used for the preparation of sera ought to be highly virulent; it appears to be of less importance whether the culture is living or dead. Pneumococci obtained by centrifuging glucose broth cultures, which have not been allowed to grow more than a few hours, provide a suitable inoculum. Broth culture, which has become dense from longer incubation, is apt to kill rabbits, especially those which have acquired a good agglutination titre.

(3) Diagnostic agglutinating sera ought to be prepared from rabbits, as such sera are highly specific. Rabbits should be injected intravenously, receiving the deposit of 100 cc. of broth culture in a series of injections on two or three successive days, followed by a rest of 5 to 7 days. This procedure is repeated for several weeks until sera with a titre ranging up to 1 in 640 or 1 in 1280 are obtained.

(4) A standard technique for the determination of type will probably be based on the methods of the Rockefeller Institute, since these are generally followed at present. For the agglutination test, a high-titred serum is used in a single dilution of 1 in 10 or 1 in 20, since there is no cross agglutination between the various types. Broth culture (without glucose) provides a stable suspension of uniform density.

The agglutino-precipitation reaction with the peritoneal washings of a mouse, which has died of pneumococcal septicaemia after an intraperitoneal inoculation, is a quick and certain test of type.

B. The classification should be uniform.

(1) The three chief types, I, II and III, defined by the workers of the Rockefeller Institute, have been generally confirmed and are accepted as standard types.

(2) The term Type IV for a heterogeneous group of strains should be abandoned, as it is liable to lead to confusion.

(3) All the strains not conforming to any of the three chief

types might provisionally be classed as Group IV, each serological type in the group being distinguished by a letter of the alphabet.

(4) The position of the American Atypical Type II strains is a subject for consideration. Two of these strains, IIA and IIB, have been found to occur in this series of cases of pneumonia next in order of frequency to Types I and II. If (3) is accepted, the Types IIA and IIB would then become Groups IVA and IVB.

C. Further work is necessary :

(1) On the distribution of types in lobar pneumonia and in other respiratory diseases. In order to obtain uniformity of classification it will be necessary for workers, through the medium of the Central Institute at Copenhagen, to make a comparison between their different strains and type sera. The Central Institute will then give the official designation to each type.

(2) On the preparation of anti-pneumococcal serum.

Is a high content of protective substances in a serum evidence of curative properties? Since a protective serum acts only upon the homologous type, its value for general use is limited. Can any method be found to widen the range of a pneumococcal serum? Is there a different principle at work in the natural cure of lobar pneumonia? The sudden amelioration of the symptoms points to a neutralisation of toxin, while the elaboration of protective substances, which can be demonstrated in a convalescent case, may insure against a relapse. Can the immunologist imitate the natural method of cure?

(3) On the action of pneumococcal vaccines in the early stages of pneumonia. Wynn states (*Lancet*, September 2nd, 1922) that early treatment with a vaccine composed of killed pneumococci greatly reduces the mortality from lobar and influenzal pneumonia.

(4) On the question of the interchangeability of type.

During convalescence from lobar pneumonia, the sputum often contains, in addition to either Type I, II or III, one or more of the atypical or Group IV pneumococci, and in the later stages of convalescence the chief type disappears, leaving only the Group IV strain. Does this indicate a change of characters on the part of the Type I, etc., or that the latter have died out and are replaced by the Group IV strain?

TABLE SHOWING THE TYPE OF PNEUMOCOCCUS FOUND IN 183 PATIENTS AND IN 10 NORMAL SALIVAS (1)

By Prof. Ernest Glynn, Liverpool

Origin of specimen of pneumococcal infection	Total No. of strains	Type I		Type II		Type II subgroup		Type III		Group IV	
		No.	percent	No.	percent	No.	percent	No.	percent	No.	percent
Pneumonias											
Lobar	105	47	44.8	22	20.9	1	0.9	2	1.9	33	31.5
Broncho- . . .	22	1	4.5	4	18.2	1	4.5	—	—	16	72.0
Empyemata											
Adults	3	2	66.7	—	—	—	—	—	—	1	33.3
Children . . .	30	12	40.0	1	3.3	—	—	—	—	17	56.7
Bronchitis, etc.	15	1	6.7	1	6.7	—	—	—	—	13	86.6
Meningitis . .	5	1	20.0	—	—	—	—	—	—	4	80.0
Miscellaneous .	3	—	—	—	—	—	—	—	—	3	100.0
Normal Saliva.	10	—	—	—	—	—	—	—	—	10	100.0
Totals. .	193	64	33.1	28	14.5	2	1.1	2	1.1	97	50.2

Annex

MEMORANDUM ON THE INFLUENCE OF IMMUNE SERUM ON THE BIOLOGICAL PROPERTIES OF PNEUMOCOCCI

By Fred. Griffith, M.B.

(*From the Laboratory of the Ministry of Health, London.*)

More exact knowledge of the natural variations in virulence and antigenic qualities which pathogenic bacteria may undergo is needed for two main purposes. In the study of epidemiological problems this information forms a necessary part of the data which should be established; and for the purpose of serum and vaccine therapy it is equally important to know in what respects immunological characters may be influenced by transfer of the bacterium from the animal body to the test tube and by culture on different media.

The conception of a "pure culture" of a bacterium as a number

(1 These investigations have been performed on behalf of the Medical Research Council and by a technique in strict conformity with the Rockefeller Standards.

of absolutely identical individuals is no longer tenable. Arkwright (1), in a recent interesting and suggestive article on the subject of variation in bacteria, with particular reference to B. *dysenteriæ* (Shiga), B. *typhosus*, B. *paratyphosus* B. and B. *enteritidis*, has shown that, in all the cultures he has worked with, two forms almost invariably make their appearance under certain conditions. These he has designated R (rough), and S (smooth), owing to the distinctive characters of their colonies on agar plates. Further important differences between the two are that each form appears to have a different main antigen, both of which are present in the parent stock; and the R form, unlike the S, does not form a stable emulsion in 0.85 per cent. solution of sodium chloride. In the bacterial species upon which Arkwright reports, the normal, at any rate superficially, resembles more nearly the S form, the R form being the "variant".

Benians (2) had previously recorded similar changes in a culture of dysentery (Shiga) bacilli.

Mary Cowan (3) has described similar varieties of colonies in cultures of streptococci; she states that "individual strains of streptococci can be 'dissociated' into 'rough' and 'smooth' types which show remarkable differences in virulence for laboratory animals".

Andrewes (4), in his investigation of the serological types in the Salmonella group, has also obtained two varieties of colonies from the same strain. In this case the colonies are identical in appearance but show differences in their immunological reactions; in the one form the antigen which evokes and reacts with the specific agglutinins is dominant, whereas the capacity to produce and react with the group-agglutinins is dominant in the other. Although Andrewes finds that the colonies of the two types are indistinguishable in appearance, there seems to me little doubt that both he and Arkwright are dealing with essentially the same phenomenon and that the differences in some of the results of the two workers merely represent different stages in the tendency of a culture to separate into individuals with distinctive characters.

The varieties of pneumococcus colonies which I am about to describe show differences in morphology and in some of their immunological reactions, which are similar to those found by Arkwright in other bacterial species. They were discovered in the course of my investigation of the mode of action of anti-pneumococcus serum. On plating cultures of pneumococci grown on homologous anti-sera, it was found that a number of colonies which differed from the normal in appearance were attenuated in virulence for mice (5).

Attenuation was definitely associated with the production of

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(1) *Journ. Path. and Bact.*, XXIV., p. 36, 1921.
(2) *Journ. Path. and Bact.*, XXIII., p. 171, 1920.
(3) *British Journ. Exper. Path.*, III., p. 187, 1922.
(4) *Journ. Path. and Bact.*, XXV., p. 505, 1922.
(5) Laura Stryker (*Journ. Exp. Med.*, XXIV, p. 49, 1916) had already observed that repeated sub-culture of Types I. and II. pneumococci in homologous serum caused marked reduction in virulence.

rough colonies, and these could be clearly distinguished from those of smooth appearance which retained their virulence.

Culture Media

Plate cultures.—Nutrient agar prepared with trypsinised meat was used, with the addition of 5 per cent. chloroformed whipped horse blood and 5 per cent. filtered horse serum. On this medium isolated colonies of pneumococci grow up to 2 mm. in diameter, and autolysis is often very slight.

The horse blood should be collected from a vessel directly into a sterile bottle, since chloroform fails to get rid of gross contamination. The blood cells, which rapidly deposit, are syphoned off into medicine bottles, and about 2 per cent. chloroform is added: it is useful to have some glass beads in the bottle to break up the cells, which form a thick mass under the action of the chloroform. The horse serum is filtered through a Berkefeld candle.

Blood broth.—Colonies from plates are grown in small tubes, 3 inches by 1/2 inch, containing about 1 cc. of equal parts of whipped rabbit blood and trypsinised meat broth. Growth occurs rapidly in the small bulk of medium, and can be recognised by the medium becoming dark purplish in colour. The blood cells keep almost intact in the ice chest for several weeks, and are useful in showing the presence or absence of hæmolysis due to bacterial growth. After inoculation and incubation, the cells fall to the bottom of the tube, and a loopful of the deposit always produces a good subculture of pneumococci in a tube containing about 6 cc. of ordinary broth.

Immune Serum.—The sera are prepared in rabbits from virulent cultures grown for 4—6 hours in glucose broth. The broth cultures are heated to 60° C. for an hour and centrifuged; the supernatant broth is syphoned off, leaving about 15 cc. out of 100 cc., in which the deposit is re-suspended. Rabbits receive 5 cc. intravenously for each injection; the injections are given in series on 2 or 3 consecutive days and at weekly intervals, and after about 2-3 months a usable serum is often produced.

For use as a culture medium the serum is distributed in small tubes in quantities of 0.5 cc., either neat or diluted as desired. A few drops of a thick suspension of red cells (rabbit) are added to favour growth and serve as an indicator.

Cultivation of Pneumococci in Immune Serum

Several serological types of virulent pneumococci have been grown in immune serum, though the majority of the observations recorded have been made upon stock strains of Types I and II, which were obtained from the Rockefeller Institute. The action of the homologous serum upon a pneumococcus culture has been controlled by simultaneous experiments with heterologous sera. After each incubation at 37° C. overnight, the serum cultures were sown on plates of blood agar so as to obtain discrete colonies.

With very few exceptions, which will be referred to later, no alteration was observed in the characters of the colonies obtained after growth in a heterologous serum, and smear preparations showed that no agglutination had taken place, the pneumococci being distributed uniformly throughout the medium. The possibility that heterologous sera produced some biological modification not associated with morphological change has not been investigated. In the homologous serum, growth took place in the form of clumps and long chains or sometimes as translucent gelatinous balls which deposited at the bottom of the tube. When a first culture in serum was shaken and plated, in addition to the normal S type of colony, a variable number of colonies appeared which differed from them in size, appearance and consistency. These were the R form. A second serum culture made from the first yielded, when plated, a larger number of variants from the normal type. After a third passage in serum the normal S form was replaced by the R. Generally the pneumococci at this stage had ceased to clump, and were tending to grow uniformly throughout the medium. When a loopful of the first culture in serum, which, as stated above, usually consists after a night's incubation of a mixture of R and S forms, is sown into plain blood broth, serial cultures up to the 13th generation have remained a mixture, the normal type in this instance showing a tendency to decrease in numbers relative to the R type.

A single colony culture of either type, made at any stage of the treatment with serum, as a rule, reproduces only the same type when sown into plain blood broth. This rule is not without exceptions, and occasionally, when the single colony culture of the variant has been made after a single serum passage, a reversion of some of the colonies to the normal smooth type has been observed.

The question of the stability of the R and S characters will be considered later.

In order to produce the variant from a normal strain through the influence of specific serum, it is necessary that growth should occur. No alteration has been observed on plating a culture that has remained in contact with the specific serum at ice-chest temperature, or has been shaken with the serum at room temperature. The variant, therefore, is a new generation in the serum and not one of the original pneumococci altered by contact with the serum. This point is of importance when one compares the action of the serum upon the pneumococci in the animal body and in the test tube.

Concentrated serum is not essential for the production of R forms. A dilution of 1 in 256 has caused a partial change of a smooth culture into a rough, even with a single treatment. In addition, the blood of a mouse, which has received a dose of 0.2 cc. of serum, has been collected about 18 hours later and used as a culture medium. The pneumococci sown into it grew as in a dilute agglutinating serum, and from one such culture, after being incubated a night and then plated, several rough colonies were produced.

Heterologous sera, as already stated, have generally no power to produce rough colonies from a smooth culture and numerous experiments have given negative results, even after several passages. I have, however, found one exceptional instance. A Type I strain grown in a Type II serum produced rough colonies when plated; these remained rough after many generations in blood broth. Until then it had seemed that the production of rough colonies in a given serum might serve as a test of the serological type. For example, the atypical II A and II B strains, which are believed by the American observers to be related to Type II, were grown in undiluted Type II serum. Both strains grew uniformly and not in chains, thus showing the absence of agglutinins for them, and after five consecutive passages in Type II serum the colonies remained of the normal smooth type. I have recorded elsewhere (1) evidence that the so-called atypical Type II strains are serologically distinct from Type II.

Description of the R and S Forms of Pneumococcus Colonies

All the serological varieties of pneumococci, with the exception of Type III, yield on the same medium colonies which are indistinguishable. There is, however, only a difference in degree between the Type III colonies and the others, and sometimes the colonies of Type II and the atypical strains of Group IV are equally large and watery. The following description of the normal type of colony applies only to strains which have been kept in a virulent condition by repeated passage through animals and maintenance in culture on favourable media. The medium upon which the special differential features of the colonies are best shown is the chocolate-coloured serum agar with chloroformed blood cells added. On ordinary agar, or on agar smeared with fresh blood, the different varieties of colonies are much less readily distinguished.

When the blood from a mouse which has died from pneumococcus septicæmia, or a young blood-broth culture, is plated on the above medium and incubated overnight, all the discrete colonies are of the same type. They are circular, dome-shaped, with a smooth shiny surface and an almost watery consistency; when autolytic changes take place they become flattened, and finally shaped like a button or draught. The characteristic feature of the variant or rough type of colony is the surface, which is dull or finely granular; the colony is more coherent than the smooth type, and may either break into pieces on being touched or stick as a whole to the point of the needle. The difference in appearance between the two varieties of colonies on the same plate is often very striking, but it is essential that the colonies should be well spaced. At first the rough variant is generally smaller than the smooth colony : it is equally bile-soluble.

(1) *Reports on Pub. Health and Med. Subjects, Ministry of Health,* No. 13, 1922.

Such are the two types of colonies which can be grown from a normal virulent strain cultivated in homologous immune serum. There is a close resemblance between the changes described above and those described by Arkwright as occurring in old cultures of various bacilli of intestinal origin. In the case of pneumococci, as with these, rough colonies can be produced also by growing cultures on agar media or in liquid media containing glucose. Arkwright, as mentioned earlier, designated his types of colonies S (smooth) and R (rough) respectively, and this seems quite appropriate for the pneumococcal colonies, the S being the normal and the R the variant. There is, however, a slight distinction, which is probably one of degree, namely, that the rough pneumococcal cultures grow uniformly in broth and form stable suspensions, whereas Arkwright's R colonies form clumps in any medium containing 0.85 per cent. salt solution. The rough cultures in broth are, however, a little more granular than the smooth; they clear more completely by centrifuging, and the deposit packs into a mass, which is not so readily emulsified as the deposit from the smooth culture.

Stability of R and S Characters

The smooth characters of the normal colonies remain unaltered so long as the strains are kept virulent by passage through animals and growth in blood broth. They persist even after prolonged culture on solid media. For example, a number of cultures were allowed to remain in sealed tubes of ordinary agar in the incubator at a temperature of 37° C. At the end of three months all were still viable; on plating, some yielded apparently all smooth colonies, and where rough colonies were detected they were few in number. A rough-colony culture, which has been produced from a virulent strain by a single passage in immune serum, may retain its rough characters for many generations in plain blood broth and, as already mentioned, may subsequently revert to the smooth type. This has occurred particularly with Type II, and the reversion in cultural characters has been associated with the recovery of virulence. Certain of the R colony strains, which have been subcultivated for many generations in plain blood broth and have been plated at intervals, have become in appearance almost indistinguishable from the S strain, but they have remained attenuated.

The change in the biological characters is of greater importance than the morphological differences between the colonies. On the other hand, the characteristics of the R type obtained by three passages through immune serum have been maintained for more than 13 subsequent generations in plain blood broth. The activity of the immune serum, *i.e.*, the height of its titre, appears to have an influence upon the permanence of the change.

Virulence

So far as my observations go at present, the typical S colony derived from a virulent culture is always pathogenic for mice.

though one cannot affirm that all S colonies are equally virulent. There is some evidence that a partial degree of attenuation is associated with a corresponding tendency to the rough state, and such partially rough colonies yield typically smooth colonies after causing septicæmia in a mouse. The typical R colony shows, on the other hand, a high degree of attenuation. When a virulent pneumococcus is grown overnight in immune serum, a plate made from the serum culture yields a mixture of R and S colonies. If a colony of each type is selected and subcultivated for several generations in plain blood broth, the S colony culture is found to be as virulent as the original culture, *e.g.*, killing mice in doses of 0·000,000,1 c.c. and 0·000,000,01 c.c. of broth culture, while the R culture has no effect in doses of 0·1 c.c. and 0·2 c.c. (the latter being the greatest amount inoculated).

Re-tested on mice after 18 generations in blood broth, the above cultures gave similar results : the R culture failed to kill mice in doses ranging up to 0·25 c.c. of blood broth culture, while the S culture had remained apparently unaltered in virulence. If a mouse dies of typical septicæmia after an inoculation of an apparently typical R culture, the colonies grown from the blood are invariably of the S type, that is, the culture has reverted. Occasionally, however, a mouse may die after a large dose of R culture, and the only organisms recoverable from the blood are a few colonies of the R type. In such a case the peritoneal washings do not give the characteristic agglutino-precipitin reaction with the type serum prepared from the normal strain, and the blood of the mouse produces no effect when inoculated into a second mouse. The following example shows how repeated passage through immune serum fixes the attenuated character. A colony of a Type III strain, virulent for mice in a dose of 0·000,000,1 c.c. of broth culture, was cultivated for one night in Type III serum; a second serum culture was sown from the first. Both serum cultures were plated when 24 hours old, and an R colony was subcultivated from each in plain blood broth. (The R colonies of a Type III strain are readily distinguished from the large watery S colonies.) Each culture was tested on mice. The R culture from the first serum killed mice, and was found on being recovered from the blood to have reverted to the S type. The colony culture from the second serum was completely attenuated, and failed to kill mice in a dose of 0·2 c.c.

Antigenic Characters of R and S Cultures

My observations have not been numerous but they show that the two types of colonies have different antigenic qualities. Sera have been prepared in rabbits with the R and S cultures of both Type I and Type II. The results with Type I sera, which I am about to describe, were also obtained with the first samples of Type II sera. Later samples of the Type II sera showed less difference between the R and S sera, and on investigation it was found that the R culture used for immunisation had partially

reverted to the S type. The Type I sera were prepared with strains which retained their special characters unaltered during the period of immunisation. Three rabbits were immunised with R culture, and one with S culture; the cultures were heated to 60° C. and were prepared freshly before each injection. All the rabbits received identical treatment, 12 injections being given intravenously during a period of five weeks. Samples of blood were withdrawn eight days after the last inoculation.

Agglutination. — The serum from the rabbit injected with the S culture agglutinated both S and R cultures up to a dilution of 1 in 160. The product of the reaction between the serum and the S culture consisted of firm masses, which could not be broken up by shaking. The R culture agglutinated with the formation of loose clumps which were readily broken up on shaking to form a finely granular suspension.

Of the three rabbits injected with R culture, one only yielded a good agglutinating serum. This agglutinated the homologous R strain up to 1 in 640, and the clumps formed were of the same loose character as those described above in the case of the reaction between the R culture and the S serum. The S culture was barely agglutinated in 1 in 10 with the R serum.

Precipitin. — The sera were tested upon broth culture which had been freed from cocci by centrifuging and filtration through a Berkefeld candle. When the S serum was mixed with the filtrate of the S culture, a precipitate was formed which aggregated into a firm mass; with the filtrate of the R culture there was no visible reaction.

The serum prepared with the R culture gave a precipitate neither with the R nor with the S filtrate.

Absorption of Agglutinin. — The serum of the rabbit immunised with S culture lost after treatment with S culture all its agglutinins, *i.e.*, both for R and S suspensions. Treatment with the R culture removed the agglutinin for the R and left unaltered the agglutinating capacity of the serum for the homologous S strain.

The serum of the rabbit prepared with the R strain required two successive treatments with the R culture before it lost the whole of its homologous agglutinin. Similar treatment with the S culture did not exhaust the homologous agglutinin from the R serum but reduced the titre for the R strain from 1 in 640 to 1 in 160.

Protection of Mice with R and S Sera. — The S serum, injected intraperitoneally into mice in doses of 0·2 c.c., protected them against a subsequent inoculation of doses of a Type I strain ranging up to 0·1 c.c. of broth culture, the virulence of which was such that 0·000,000,01 c.c. of broth culture killed an unprotected mouse within two days. Against the same strain of Type I, all the R sera failed to show any protective power. The inoculation of the three rabbits with R culture was continued in order to ascertain whether more prolonged treatment would induce the

development of antibodies against the virulent strain. The three rabbits were re-tested after they had been immunised for a total period of four months, during which each rabbit received 26 intravenous injections. Their sera still failed to show the presence of protective substances when tested against a Type I culture which killed mice in a dose of 0·000,000,01 c.c. Two other rabbits, which had been injected with the S culture for five weeks and were tested at the same time, yielded sera which protected mice against doses of 0·1 c.c. of the above culture.

Influence on the Type of Colony by Growth in R and S Sera

A virulent Type I culture was grown in R serum and in S serum. There was no alteration in the character of the colonies obtained on plates after three successive passages through the R serum. On the other hand the culture grown in the S serum, when plated after incubation overnight, yielded a mixture of R and S colonies. One of each variety was subcultured in plain blood broth and was tested for virulence on mice. The S strain killed mice in a dose of 0·000,000,1 c.c. of broth culture, while 0·2 c.c. of the R strain proved innocuous.

Agglutinability of R and S Strains

The clumps formed after the interaction between a virulent pneumococcus culture and homologous immune serum are very characteristic. On the addition of a 1 in 10 dilution of the serum to pneumococcus broth culture there is an immediate turbidity, and visible clumps quickly form, especially on gentle shaking, which aggregate into a compact mass. An almost identical result, but a less bulky precipitate, is obtained with a broth culture from which the cocci have been removed by filtration. Thus, this type of reaction, which is very specific for each serological type of pneumococcus, depends on the formation of a precipitate between the serum and a soluble substance in the broth. If, however, the centrifuged cocci from a broth culture are resuspended in salt solution, they form a compact mass with immune serum similar to the above, although the soluble substance has been removed with the broth. This appears to indicate that either the external portion of the pneumococcus contains some of the soluble substance in process of secretion or both are of similar constitution.

The attenuated R strain agglutinates with the same serum in a different manner. If broth culture is used, agglutination is slow, and fine granules form which settle to the bottom of the tube. A more copious deposit is formed when a thicker salt solution suspension of cocci is used. In both cases, however, the clumps do not aggregate into a compact mass but can be readily shaken up into a finely granular suspension. The difference in character between the reactions of the R and S strains seems

capable of a simple explanation. Since no precipitation or turbidity occurs when the serum is added to a filtered broth culture of the R strain, it is obvious that the R culture does not form any soluble substance, and hence there is nothing to bind the agglutinated cocci into a firm mass.

The R culture suspensions are rather less stable than the smooth and, in addition, they are less specific in their agglutinability, often showing some reaction with heterologous sera; they continue, however, to react with the type serum to a higher titre than with any other pneumococcal serum.

Mode of Action of Protective Anti-Pneumococcus Serum in the Animal Body

The attenuation of virulent pneumococci by growth in homologous immune serum *in vitro* is a phenomenon which is probably related to the protective action of the serum in the animal body and may help to explain it, though the two processes are obviously not identical. In the test-tube the action of the serum is shown in the altered virulence of the new generation of pneumococci, though the change is never complete in the first passage through serum, the culture remaining a mixture of virulent and non-virulent elements. Though pneumococci have been recovered from an immunised animal at several stages before their final disappearance, it has not been possible actually to demonstrate any change into rough colonies. In an immunised animal the inoculated pneumococci have generally disappeared from the peritoneal cavity within 24 hours; they remain alive longer when inoculated into the subcutaneous tissues, where they give rise to a localised abscess.

The following is the record of an unsuccessful attempt to demonstrate the production of rough forms of pneumococci in an immunised mouse. A virulent Type I culture in broth was mixed with an equal quantity of a Type I serum and incubated for half an hour, after which the mixture was inoculated intraperitoneally into two mice. A small amount of peritoneal fluid was withdrawn from each mouse one and two hours later, part of which was sown on plates. Only smooth colonies of virulent type grew, but mice inoculated with the rest of the fluids remained well. The two original mice were killed 20 hours after inoculation and the peritoneal cavities were washed out with salt solution. The centrifuged deposits from the washings were sown on blood agar plates; no growth of pneumococci was obtained.

If there is an intermediate stage in the mechanism of protection during which rough attenuated pneumococci, that is, cocci which have not developed the part of their external structure associated with the secretion of soluble substance, are produced in a passively immune mouse, it is clear that they would readily be destroyed, since even large doses of these R cocci inoculated into an unprotected mouse quickly disappear.

On the other hand, it may not be necessary to postulate such

an intermediate stage. The immune serum may protect the mouse by depriving the pneumococcus of its virulent potentialities, possibly by precipitating the external covering associated with capsule formation and the secretion of soluble substances. The pneumococci so affected would become the prey of the phagocytes. They would, if removed from the animal before destruction, be capable of multiplying, and as the exposure to the immune serum had been brief, they would produce normal virulent descendants. But if within the animal body some sensitised pneumococci escaped the attacks of the leucocytes and divided, the descendants would remain under the influence of the serum, in which case the property of the serum to produce progressive attenuation might be brought into action.

Discussion of Results

1. The first point of interest which emerges from this study of S and R forms of pneumococci is the fact that a definite morphological criterion has been found to distinguish between virulent and attenuated strains. Further evidence of attenuation is furnished by serological reactions, in particular by the loose character of the clumps in the agglutination test, and by the absence of specific soluble precipitable substance. Given these data, it will be possible to decide whether a culture is in the condition suitable for the production of disease.

2. An important practical corollary will be that, for the production of good immune sera, strains should be used which consist entirely of S forms. Immunisation of rabbits with the R form has up to the present stage of the investigation failed to stimulate the production of protective substances against a virulent strain, whereas in the same time the injection of the S form produced a strongly protective serum.

3. As regards the influences which cause attenuation of virulence, I have found that this result can be produced invariably by growth in specific immune sera. I have also noted that R forms occasionally appear after prolonged residence on ordinary solid media. It is possible that similar changes may arise under natural conditions, as, for example, during convalescence from pneumonia. The study of the various influences which cause exaltation or attenuation of virulence may be facilitated by observing the occurrence of S and R colonies under different conditions.

4. This alteration from an S to an R type appears to be a natural tendency with many species of bacteria. It can, I think, be attributed to degenerative changes and is associated with the loss of certain antigenic qualities. For example, while studying the serological types of meningococci (1), I subcultured separately 40 colonies of identical appearance and tested them against an agglutinating serum prepared with the whole strain. With one

(1) *Journal of Hygiene*, XVII., p. 166, 1918.

exception, all the daughter colonies absorbed the homologous agglutinin. The exceptional colony, although it agglutinated as well as any of the others, failed to lower the titre of the serum for the parent strain. This last result I considered to be evidence of a diminution of antigenic complexity. Now the relationship of the R pneumococcus to the S is in some respects similar to that of the variant meningococcus to the whole culture. A serum prepared with the stock culture or with the S form of pneumococcus agglutinates both R and S to the same titre. The S form removes the whole of the agglutinin both for R and S, but absorption with the R form has no effect on the titre of the serum for S. The S and R possess related antigens, as is shown by the agglutination test, but the R lacks an antigen which is contained in the S. Before it can be said that the R is simply an S form from which part of the latter's antigen has been removed, it must be shown that the R type of antigen is contained within the S form. A serum prepared with R and agglutinating that form up to 1 in 640 barely agglutinates the S form; if present in the S form the R antigen therefore is not available, or its union with the R antibody does not result in agglutination of S. Still, repeated absorption of R serum with S reduces somewhat the agglutinin for R, thus indicating that there are receptors of the R type present in the S.

5. What are the distinguishing features of the part of the antigen of the pneumococcus which is concerned in virulence? As one would naturally expect, in view of the intimate association of a capsule with virulence, it appears to be the outer portion of the pneumococcus which is modified in the change from S to R. After growth in immune serum the virulent pneumococcus colony loses its smooth shiny surface and almost watery consistency and becomes dull and finely granular. That is to say, the individual pneumococci tend to adhere and are not separated by some substance which, probably on account of its being soluble in salt solution, permits the formation of a very fine stable emulsion. In addition, the attenuated R form no longer secretes in young broth cultures a soluble substance which reacts with an anti-serum from a virulent strain. There is some evidence, therefore, that the R form, which is undoubtedly derived from the S, is differentiated from the latter by the loss of that part of its antigen which is concerned with virulence, capsule formation and secretion of specific soluble substance. The R form may be considered to be a stage in the degeneration of a pneumococcus from a virulent complex type to an attenuated form with a simpler antigenic structure.

6. The next observation of interest is that this loss is not necessarily permanent. Though in certain cases, as in old cultures and after repeated serum treatment, the R forms are apparently stable, it has been found, under other circumstances, that this alteration in an important biological function can apparently be reversed, not only by animal passage but also by continued subculture in blood broth. After many generations, the R

pneumococci assume again their smooth colony formation and at the same time their virulence is restored. Can it be said, in explanation of such results as these, that the original R colony contained a few virulent pneumococci which escaped notice in the earlier subcultures? This hypothesis, in view of the negative results of the periodical tests for virulence, cannot be regarded as probable. Yet it is difficult to understand how an attenuated organism can recover its virulence simply by repeated subculture without animal passage. A somewhat similar event has been described by Bail (1) in his work on the formation of capsules by B. anthracis. If a culture of anthrax bacilli is exposed to a temperature of 42° C. for a period which falls short of depriving it completely of the power to produce capsules, certain strains are formed which produce a mixture of colonies. Some of these colonies produce cultures all of which invariably fail to form capsules, while others produce in addition a few typical capsule-forming bacilli. This result, he thinks, depends upon a deficient inheritance of the capsule-forming substance, so that an individual bacillus is able to endow only one of its descendants with a sufficient amount to produce a typical capsule-forming strain.

7. Does this investigation throw any new light upon the protective action of pneumococcus immune serum? According to Neufeld's bacteriotropic theory, the pneumococci after inoculation into an immunised animal become sensitised and are then destroyed by phagocytosis. Experiments *in vitro* provide supporting evidence, since virulent pneumococci are not ingested by leucocytes except in the presence of immune serum, and the leucocytes are thought to play an essential part in the mechanism and not to act merely as scavengers.

Perhaps the above observations on the attenuating action of immune serum may serve to amplify Neufeld's theory, the suggestion being that the action of immune serum is not entirely bacteriotropic. A certain number of pneumococci may not be directly subjected to phagocytosis in the presence of immune serum. These may give rise to new generations in the animal body, but such bacteria would, under the influence of the immune serum, be subjected at the onset of growth to rapidly progressive attenuation and would soon be destroyed.

Summary

1. Virulent pneumococci become attenuated by growth in homologous immune serum.

2. The loss of virulence is associated with an alteration of the immunological characters.

The attenuated pneumococcus does not stimulate the production of protective substances in the blood of inoculated rabbits.

3. There is a morphological distinction between the colonies

(1) *Centralbl. f. Bakt.*, Orig., LXXIX., p. 425, 1917.

of the virulent and the attenuated pneumococci. The virulent colonies are smooth and the attenuated rough in character, the distinction being similar to that between the R and S varieties of B. dysenteriæ, etc., described by Arkwright.

4. The S pneumococcus produces in young broth cultures a specific soluble substance; the R pneumococcus does not.

5. The S form agglutinates with specific serum, producing a firm gelatinous precipitate; the R form produces loose clumps which are readily shaken up.

6. An R strain may revert in all respects to the S type, or may remain unchanged after many generations in subculture in plain blood broth. On the other hand, the morphological distinction between the R and the S forms may tend to disappear, while the immunological differences persist.

7. The attenuation of pneumococci by immune serum in the test-tube indicates that the mode of action of the serum in a protection experiment is a direct one upon the pneumococcus.

A double action may be suggested :

(*a*) The serum may disorganise the biological functions of the pneumococcus by precipitating the capsule, thus inhibiting the secretion of anti-leucocytic substances and rendering it temporarily harmless.

(*b*) When pneumococci divide in the animal body in the presence of immune serum, it is suggested that the influence of the serum may cause progressive attenuation of subsequent generations.

November 10th, 1922.

INVESTIGATIONS WITH REGARD TO PNEUMOCOCCI

By F. Neufeld

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I. We have so far collected little new material with regard to the occurrence of the various types present in cases of pneumonia in Germany. A report has already been published on a number of cases which occurred in 1912 and 1913 (*D. med. Wochenschrift* 1922. No. 2). Further investigations are being made, and different parts of Germany will be considered.

II. As regards the titrating of sera, experiments were made on mice for the purpose of determining the comparative value of various methods. Special attention was given to the method employed in America (intraperitoneal injection of 0.2 serum mixed with increasing quantities of culture) and to the method applied by Neufeld and Händel (intraperitoneal injection of graduated quantities of serum and, three hours later, moderate quantities of culture, also injected intraperitoneally). Both methods appear to give results which are accurate and in fair agreement with each other. Further investigations are required to determine which of these methods permits of the more exact gradations. At the same time other methods were examined in which the serum was introduced partly intravenously—but principally subcutaneously—and the culture introduced twenty-four hours later into the peritoneal cavity. Under these conditions, in which the serum (as is the case when it is used on human beings) is not applied to the *seat* of the disease but finds its way there by means of the blood stream, absolutely different quantitative results are obtained. In this case the so-called "Schwellenwert" (stimulation threshold) is clearly apparent, whereas the action of the serum in the methods previously mentioned *approximately* follows the law of *multiples*.

Recent papers frequently emphasise the importance of arriving at a consensus of opinion as to the nature of the action of the serum. It is certain that agglutinins have no share in this action, and it has been proved, more especially by Barber (*Jour. Exp. Med.*, Vol. 30, p. 30), that the serum does not kill the cocci or check their development. For my own part I have assumed that bacteriotropins are the active substances, but this hypothesis has been disputed since the protective power of the serum is not supposed strictly to correspond to the quantity of bacteriotropins present. If there are samples of sera which clearly possess protective power but do not produce phagocytosis—and I am not aware of any accurate observation on this subject—different laboratories should be given an opportunity of testing these sera. The researches of Cole and of Dochez and Avery (*Jour. Exp. Med.* Vol. 26, pages 453 and 457) prove that considerable quantities of dissolved substances which fix the protective substances of the serum appear

in the blood of human beings and animals infected with pneumococci. Whether these substances perhaps are of the nature of endotoxins—which are neutralised by anti-endotoxins—is a question which can only be decided by further investigation.

Naturally, the investigation of the anti-pneumococcus serum is of special importance inasmuch as it may lead to securing a useful curative serum, but it is of paramount importance that further experiments should be made with regard to the curative power of the serum in cases of pneumonia, the more so as the results recorded by Cole and his collaborators have been challenged even in America by Thomas. For this purpose fairly large quantities of horse serum of high potency have been prepared in a German factory under my supervision. This serum, on being tested and compared with the curative serum of the Rockefeller Institute, was found to be of identical value. Its action will be tested on patients in the near future.

III. We deemed it necessary to re-examine the basic principles on which types were determined. We have, moreover, made thorough comparative investigations by employing various methods of protective inoculation and various methods of preparing the serum. The experiments which were mainly carried out by Dr. Yoshioka in Dr. Schiemann's laboratory will shortly be published in the *Zeitschrift für Hygiene* (1). The chief results so far are as follows :

(1) Under conditions which, as is shown by experiment, adversely affect their virulence (such as incubation at a temperature of 39°, incubation on the surface of a poor nutrient medium exposed to the light, excessive or too slow drying in the desiccator), pneumococcus strains can easily be modified agglutinatively; however, there is no direct relation between the loss of virulence and the serological modification. Such strains more or less completely lose their power of agglutinating with sera of their own type, and thereupon acquire the property, though irregularly, of agglutinating with sera of foreign types and even with streptococcus sera. On loosing their virulence, streptococci may also undergo complete serological change and agglutinate with pneumococcus sera.

(2) Sera produced with typical pneumococci are generally believed to be strictly specific for the type concerned. But when we investigated the sera of immunised rabbits soon after the bleeding, we found almost invariably that they also influenced other types—frequently very slightly, but sometimes more powerfully than their own type. These heterologous agglutinins are not destroyed at 56; but *disappear* if the sera stands for a few weeks, while the homologous agglutinins remain quantitatively the same.

These sera are, on the other hand, strictly specific in protective tests, thus supplying a further proof that agglutinins have no share in the protective value of the serum. Observations made

(1) Yoshioka : *Beiträge zur Pneumokokkenimmunität*, I-V; *Zeitschr. f. Hygiene und Infektionskrankheiten*, Vol. 96, 1922 and 97, 99, 1923.

by Blake and Cecil on monkeys show, however, that heterologous protective substances may also make their appearance occasionally.

(3) Observations by Blake and Cecil of the effects produced on monkeys, and by Cecil and Austin of the effects on human beings, favour the view that a "crossed" immunity may appear as a result of active immunisation to a certain degree. The inoculations which Lister carried out in South Africa, however, showed contrary results.

The results of our experiments on the active immunisation of mice showed a distinct, though a considerably weaker, immunity against heterologous types. Whether this immunity is of shorter duration than the homologous immunity is a matter which requires further investigation.

The above-mentioned investigations into the serological modifications of pneumococci appear to me to be of great practical importance and worthy of being carried further, especially for the following reason. Although such a variation has not as yet been produced by experiments, the observations which have been made may perhaps be held to point to the possibility that typical "fixed" pneumococci may originate from the cocci present in normal throats. This question is one of paramount importance in the epidemiology of pneumonia. Epidemiological and experimental observations must be carried on side by side if this problem is to be solved.

(4) In numerous experiments, made chiefly on mice, tests were carried out for the purpose of comparing the action of protective inoculation with dead virulent and avirulent pneumococci, with living avirulent pneumococci and with living virulent pneumococci mixed with serum.

Avirulent dead cocci had exceedingly little protective value; avirulent living cocci also gave less satisfactory results than dead virulent cocci. In vaccinating with pneumococci (and other bacteria) more attention should be paid than has hitherto been the case to use as virulent cultures as possible. Many experiments and analogies support the view that pneumococci which are no longer virulent in mice must have lost their virulence for human beings also. The converse cannot be affirmed without further investigation. Naturally we cannot test the virulence in the case of a human being but can merely test the antigen action. I therefore think it highly desirable that further observations should be made with regard to the occurrence of protective substances in human beings after inoculation with various pneumococcus strains, and, especially, with strains which were isolated a short time before from a human subject but which are virulent only to a slight degree.

It would, moreover, be highly desirable to continue the investigations of Cecil and Austin and Lister and to make further researches regarding the dosage and the interval for protective inoculation in human beings. Experiments on mice convinced us of the importance not only of the total dose but also of the manner in

which it was divided up into single doses. These experiments showed, for example, that the dead cocci which were contained in 0.3 and 0.03 bouillon culture had much greater protective power than a quantity ten times and one hundred times greater; and that, moreover, the division of the entire quantity into doses for six injections administered within three hours gave better protective results than injections of the same dose spread over several days or weeks.

(5) Our experience goes to show that in producing curative serum the greatest importance should also be attached to the virulence of the cultures. We obtained, at least on rabbits, unsatisfactory sera with large doses of dead cocci— a result which confirmed the statements of Cole and Moore. Here also a great deal appears to depend on the correct interval. For example, we obtained from a rabbit which was given six intravenous injections during a single day and on which this treatment was repeated twice at a week's interval, a serum which had the same protective value as the best horse sera. It is therefore not impossible that sera of still greater potency may be obtained from horses as a result of special dosing and the careful arrangement of the time interval of the doses, and it is certainly desirable that institutes which have adequate resources for the purpose at their disposal should conduct experiments along these lines.

IV — ANTI-DYSENTERY SERUM

STANDARDISATION OF DYSENTERY SERUM

REPORT

By Professor Doerr

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The Health Committee of the League of Nations placed—among other items—on the Agenda of the Conference which it convened in London in 1921, *the problem of a uniform method of testing the potency of therapeutic dysentery sera.*

The necessity of arriving at some international agreement is as obvious in this case as in the case of any other serum used for therapeutic purposes. This necessity would only disappear in case anti-dysentery serum should cease to be of value as a therapeutic agent in the treatment of dysentery.

Such is, however, by no means the case at the present moment. It is true that medical literature—especially the literature of the war period—contains a considerable number of reports and notices which comment *very unfavourably* on the results obtained by serum treatment in cases of bacillary dysentery, or which argue that, although it has been proved to exercise a beneficial effect on the course of the disease, results of equal value—both as regards quantity and quality—could be obtained with *normal horse serum* (1). These unfavourable conclusions are, however, counterbalanced by other reports, based on a large number of recorded observations and setting forth results which would justify us in assuming that the serum, and, *in particular, its specific components,* will play an effective, indeed an indispensable, role in the treatment of dysentery, at least in the near future.

It is not, however, possible to form a reliable and correct judgment in regard to these opposing views, *because the immense majority of the reports concerning results of serum treatment of bacillary dysentery are absolutely valueless for purposes of comparison.*

The sera which the authors used for therapeutic purposes were either not described at all or described very inadequately in so far as the technique of preparation and the *amount and potency* of their *specific components* are concerned, and in many cases information in regard to the ætiology of the cases of dysentery treated with the sera is lacking or incomplete. In such circumstances it would be premature to side with either of the extreme opinions; least of all

(1) C. Singer.

would it be advisable to reject the favourable reports and to attribute the opinions expressed therein to certain sources of error (*e.g.*, to over-optimistic estimates or to biased statistical deductions from the recorded observations, or again to the mild nature of the epidemics in question). From an objective point of view, the situation in regard to the real value of serum treatment in cases of bacillary dysentery may be stated as follows : excellent results obtained by anti-dysentery serum have been reported by so many reliable and independent observers that it appears justifiable, for the present, to conclude that this serum has a beneficial therapeutic action. *On the other hand, we know very little as to the conditions under which this capacity may be developed to its utmost extent;* indeed, to be perfectly objective, we know little more than *that such conditions exist,* for experience shows that serum treatment has, as a fact, *failed* in a very large proportion of cases of bacillary dysentery.

In spite of this cautious and non-committal statement, we may answer in the affirmative the question as to whether serum treatment should maintain its place among the therapeutic methods of treating bacillary dysentery, and this conclusion, of course, leads us to admit, by implication, the necessity of standardising the methods of measurement of dysentery sera.

It must, however, be recognised that this standardisation is not solely designed, as in the case of diphtheria and tetanus serum, to provide physicians with a guarantee for the quality of the serum and to safeguard them and their patients against worthless productions, but that it is primarily intended to assist us in the solution of another problem, *i.e., it must furnish us with reliable and scientific reasons, on a wider and more accurate basis than heretofore, for believing that serotherapy is of assistance in the treatment of dysentery.* The only way to remove any remaining doubts (1) and ambiguities in this connection is the method which I proposed more than fifteen years ago, and which was unfortunately neglected : *known cases—i.e.,* cases of dysentery from ascertained etiology —must be treated with *known sera.* The word "known" in the sense here intended can only be applied to anti-dysentery serum if the whole of its specific relations to the various types of dysentery bacilli have been determined both quantitatively and qualitatively, or if at least one of these relations which, by reason of the particular method by which the serum has been prepared, may be regarded, *a priori,* as *predominant,* has been tested by some internationally recognised standard and by a method which ensures constant results. The more accurately and extensively these tests are carried out, the more light will be thrown on the whole group of problems connected with the serum treatment of dysentery, and *I therefore urge that the Health Committee of the League of Nations* should organise an enquiry with this object in view and

(1) These include : the co-ordination of determined types of serum with determined etiological forms of dysentery, the problem of monovalency or polyvalency, the importance of both anti-infection as well as antitoxic serum contents and the definitive elimination of all types of bacillary dysentery which are not capable of being influenced by serotherapy.

should entrust specialists of repute with the application of the therapy and with the bacteriological investigations, and should designate a well-qualified serum institute to prepare and test the sera necessary for this purpose.

I still adhere to this proposal, the reasons for which —so far as I am aware—have been in no way modified since the London Conference.

The situation is different so far as regards *the measurement of anti-dysentery sera* as such, and in particular *the technical aspect of the process.* At the time when the Conference was convened, the bases for conclusions which would secure international recognition did not exist, at any rate not in such a form as would have made it possible to establish a uniform method of procedure which would have secured undisputed recognition by the serum institutes, as the best obtainable at the moment, and which might be expected to remain valid for some time. This state of affairs must have been appreciably improved by the experimental investigations carried out in pursuance of the Committee's instructions, perhaps even to such a point that it might now be possible, with some hope of success, to commence a discussion regarding the methods of standardisation; at present, however, I have no information at my disposal on this subject. I must content myself with outlining the situation as it existed *before the Conference* referred to and formulate proposals on the basis of the facts which have been established. A survey of the work of those who took part in the Conference will show to what extent these proposals have already been carried out and what remains to be done.

In order to realise the difficulty of standardising the methods employed for testing the potency of dysentery sera, *it is only necessary to limit the problem to the simplest possible case* (regardless of whether this simplification appears admissible or not from a theoretical standpoint or with regard to the practical requirements of serum treatment in the case of dysentery) and to consider whether, even on the basis of these almost purely fictitious premises, it would be possible to come to an immediate decision as to the most suitable form of test.

Now the simplest case, as regards anti-dysentery serum, would obviously be that in which we *merely had to determine the amount of antitoxin which it contained.* "Antitoxin" in this connection must only be understood as meaning the antibody *which neutralises the toxin of the Shiga-Kruse strains.* Toxin-antitoxin titration offers a well-established and, as regards diphtheria and tetanus toxin, a very carefully explored field of research, so that there is good reason to hope that the experience gained therein may also be of use with regard to dysentery serum; from a technical point of view, the cultivation of virulent Shiga-Kruse strains and of sufficiently concentrated toxic solutions presents no difficulties, so that, in this respect also, the way appears clear. On closer examination, however, the problem of finding a satisfactory method of titration of the antitoxic components of dysentery serum is much more complicated.

As long ago as 1907 I pointed out that bouillon culture-filtrates of Shiga-Kruse bacilli could be so far modified by heating that they wholly or partially lost their effect on the nervous system of rabbits, whereas the usually very variable changes in the cæcum and the large intestine were found to be more regular and more marked. I suggested as a possible explanation that the toxic solutions may have contained two components, a thermolabile *neurotoxic* component and a relatively thermostable entero-toxic component. This problem was further investigated by *Olitsky* and *Kligler* at the Rockefeller Institute in 1920 (without, however, making any reference to my researches) and they came to the conclusion that the Shiga-Kruse type of dysentery bacillus produces two toxins, a thermolabile toxin, which is only found in filtrates of quite fresh bouillon cultures, and a thermostable entero-tropic endotoxin, liberated when the bacterial cell dies and desintegrates—these two toxins have two corresponding different anti-toxins, the *anti-exotoxin* and the *anti-endotoxin*. To judge by the few published records, the entero-tropic endotoxin cannot be neutralised by a purely anti-exotoxic serum, so that in mixtures of exo- and endo-toxin, it is possible to separate the two toxins by means of elective saturation, a procedure which, in its results, is equivalent to the "heat-separation process" which I was the first to describe. In the *pathogenesis of dysentery symptoms in human beings* (or, more correctly, in the development of the symptoms of Shiga-Kruse dysentery), the leading part is played by the entero-tropic endotoxins—to use the terminology of Olitsky and Kligler—in the first place, and in a lesser degree, or not at all, by the neuro-tropic exotoxins—or it may be that these substances act upon the central nervous system of human beings in a different manner from that, and to a lesser extent than in the case, of rabbits : it is, however, beyond dispute that changes in the intestine are a predominant symptom, and this fact must be taken seriously into account (if the hypotheses of Olitsky and Kligler are correct) both in the production of serum and also when estimating the neutralising properties of therapeutic sera. But is the hypothesis of the two above-mentioned authors based on adequate grounds? I think that this question must be answered in the negative, and this leads us automatically to demand that the complex nature of the Shiga toxins should be submitted to further research in the course of which it should be possible to estimate the importance of the investigations of Horimi, Fukuhara and Ando, whose fundamental ideas are rightly disputed by Pribram and others. In carrying out the necessary investigations, we might also hope to obtain some information regarding the manner in which the Shiga toxins act, for the remarkable specific action on organs of these toxins have always been adduced as evidence indicating the existence of several different toxins, although these phenomena are susceptible of a different explanation.

The measurement of dysentery sera, even in its simplest form, namely by the titration of Shiga antitoxins, necessitates the solution of theoretical questions of essential importance; it would

likewise involve *a whole series of technical details*. There is not even agreement as to whether *rabbits* or *white mice* react more regularly to the toxins of the Shiga-Kruse bacilli; it would also be necessary to ascertain, in the case of white mice (if these be the animals selected), whether preference should be given to the intraperitoneal injections recommended by Kolle, Heller and Mestral or to the intravenous method of injection proposed by Sachs and Georgi. Moreover, it is not known whether the susceptibility of rabbits and white mice to all Shiga toxins is constant, or whether it varies in the case of different toxin solutions. Finally, it should be decided whether the toxin and the anti-toxin are to be injected as a mixture or separately. Kraus, like myself, raised the objection that, in the case of injection of the toxin-antitoxin mixture, the procedure was not comparable to the method in which the serum is used for therapeutic purposes, and that a closer approximation to the conditions under which the serum is used in the treatment of patients can be attained if the toxin and the serum are injected not simultaneously, but separately in an animal susceptible to the toxin. In this way the results are very different from those obtained by the titration of toxin-antitoxin mixtures *in vitro*. Kraus urged, in this connection, that antitoxic dysentery sera should only be allowed to be sold if 0.1 cc. of this serum injected intravenously is able to neutralise a single fatal dose (or in certain conditions a double or even a four-fold dose) of toxin injected just previously into the auricular vein on the opposite side. That is, however, as I am now quite willing to admit, not a method of measuring the potency of the serum but merely the definition of a minimum standard, *the determination of a minimum limit*, and of a standard which is not sharply defined, since the *interval of time between the injections of the toxin and of the serum*, which may have the greatest influence on the fate of the injected animal, cannot be exactly prescribed and also because the term "a fatal dose" is not clearly defined. Moreover, it does not appear to be by any means certain, *a priori*, that the method introduced by Kraus and still retained by the Vienna State Serotherapeutic Institute, for determining the "separate value" has any connection with the "curative" capacity of the sera, or with the degree of "avidity" of the antitoxin in question for the toxin. Moreover, this question is not yet ripe for discussion, and requires further systematic investigation.

If there were general agreement regarding the technical methods of estimating the potency, we should be confronted with a new problem, that of preparing and maintaining a standard not liable to variation and capable of being reproduced. Fluid sera differ, as both Kraus and I have pointed out, from the very outset, and so far as I am aware, no form of the toxin has been discovered which would be sufficiently constant to be suitable for this purpose. It will therefore be advisable to adopt the technical method of the *dry standard serum*, such as *Ehrlich* introduced for testing the potency of diphtheria sera.

This method of procedure has indeed been adopted in several

institutes, for example at Frankfort-on-the-Main and at Osaka (Japan). In *Osaka,* in particular, the procedure, according to *Fukuhara* (*Journal of Immunology,* Vol. IV, 1919), is that a dry standard serum is kept if its antitoxin unit, arbitrarily selected, was still able to neutralise 100 minimum lethal doses of a particular toxin (the mixture being intravenously injected into the auricular vein of rabbits). With the help of this unit the Lo and L+ dose in any toxin is determined, and the latter is defined as the toxic mixture which, when added to an anti-toxin unit of standard serum, kills rabbits weighing 1,500 to 2,000 gr. in 4 to 5 days. The sera are titrated with the L+ dose. As will at once be seen, *Fukuhara's* object was to obtain as faithful a copy as possible of the Ehrlich method for testing diphtheria antitoxin. The records of *Fukuhara's* experiments, however, in no way vouches for the efficacy and precision of his method when applied to antitoxin dysentery serum. Moreover, the reasons advanced by *Ehrlich* would seem to be inapplicable here, for *Fukuhara* himself admits that the deterioration of the dysentery toxin effected by keeping converts the toxin molecule into a non-toxic modification *which does not possess neutralising power.* In other words, *unlike the case of diphtheria toxin, the formation of "toxoids"* does *not take place.* This being so, it will be possible to effect a very considerable—and very welcome—simplification in the measurement of the potency of the antitoxin. On this subject, however, considerable investigation will be necessary before a satisfactory solution can be arrived at, the object of such studies being to determine the *toxicity of the various Shiga toxins and their power to neutralise antitoxic sera.*

We shall now return to the starting-point of our discussion and endeavour to ascertain whether this apparent simplification of the general problem of the determination of the potency of dysentery sera has actually been realised in theory and practice. Everyone acquainted with the subject will agree that there is no question here of titrating any particular property of dysentery sera, but rather of measuring what property or properties are essential to the therapeutic efficiency of the sera or may at least be assumed to contribute to this efficacy. Practising physicians and doctors attending hospitals are apt to conclude from the statement that a therapeutic serum is "of high value" that it is of high value in the sick-room, and when they find their expectations always, or at least often, disappointed, they attach no meaning to the indication of value; they declare it to be useless. In consequence they either give up serotherapeutics altogether, or else lay down for their own use certain empirical principles (such as dosing by volume instead of quantivalency, identification of serotherapeutics with parenteral protein-therapeutics, preference for specific sources of supply), and act in the light of these principles. I consider this to be a source of serious danger, owing to the fact that standard units are fixed without any sufficient investigation of the question as to *what should really be measured.* I consider that these dangers should be counteracted by the enquiry

which I proposed in the first instance, having for its object a determination of the serological and other conditions influencing the therapeutic value of sera in the treatment of dysentery patients.

From this point of view we should be acting arbitrarily if we were to limit ourselves to the production and titration of the antitoxins for the Shiga-Kruse toxins, for Pribram, Wassermann, Ficker, Sonne, Thiotta and Sundt (See *Journal of Immunology*, Vol. VI. 1921) have also obtained toxins from mannite fermenting bacilli which differ from the Shiga-Kruse type, in regard to their agglutination properties—these toxins produced paralysis and intestinal lesions in white mice and had specific corresponding antitoxins differing from the Shiga antitoxins.

Moreover, it is still uncertain what attitude we should adopt in regard to the so-called bactericidal properties of the dysentery sera. The historical evolution of the question was as follows : Kruse (who experimented with *guinea pigs*, which are not susceptible to the Shiga toxins, naturally made use of the well-known peritoneal experiment for testing the anti-dysentery sera; this was perhaps natural in view of the opinions then prevailing as to the immense importance of bacteriolytic antibodies in immunity. When, later on, Todd, Rosenthal, Kraus and I had demonstrated the susceptibility of rabbits to the toxin of the Shiga-Kruse strains, various methods were adopted for the manufacture of dysentery sera and for the titration of such sera. But when efforts began to be made to adopt the serum treatment in cases of dysentery caused by atoxic (deficient in toxin) strains of bacilli, there was a reversion to the original method of preparing and testing anti-infectious sera.

The exigencies of practice finally led to the fusing of both the principles of serum production and serum testing. The requirement that every case of dysentery should be treated with an antiserum specially prepared as an antidote to the bacillus which had caused it, or the toxins of that bacillus, had been found to be most inconvenient, in fact, impracticable. The bacteria responsible for an attack of dysentery cannot be ascertained in a considerable percentage of cases, and, in many others, not with the speed required for successful serotherapy. In the conditions that prevailed during and since the war, many epidemics of dysentery presented cases caused by various dysentery bacilli : homogeneity in this matter was the exception rather than the rule. Thus it was that "polyvalent sera" were prepared by immunisation with Shiga-Kruse cultures and various atoxic types of bacilli, and these were tested in the most various, and sometimes the most arbitrary, ways. The method of titration employed by Flexner and Amoss must be regarded as the best then known for such "polyvalent sera". The authors measured the strength of the anti toxin by means of a "mixed" intravenous dose injected into a rabbit of 1250 to 1500 gr. and they determined the bactericidal power by tests on guinea-pigs of 225 gr., by means of intraperitoneal injections of a mixture of living Flexner and Shiga bacilli suspensions with serum.

Whether the estimation of bactericidal power, as well as of

the antitoxin content of serum used in the treatment of Shiga infections, is of any value, or whether bactericidal properties of sera are of value in the treatment of cases caused by atoxic bacilli (Kruse would actually like to exclude all these cases from serum treatment): or whether again (as Pribram believes) a purely anti-toxic serum also exercises a favourable influence even on cases infected with atoxic bacilli—all these are questions which are still undecided. They are, however, all questions which must be settled before we can bind ourselves for a number of years, by international agreements, to standard units of measurement, methods of measurement, and methods of production.

Before concluding these remarks, I should like to point out that the serotherapy of dysentery has been very considerably affected by the discovery of bacteriophages by *d'Herelle*. I consider it improbable, however, that serotherapy will be completely superseded by the enteral or parenteral infusion of bacteriophages intended as an antidote for any bacillus which may have caused dysentery. Nevertheless, any comprehensive action, aiming at elucidating the whole of the facts which bear upon the serotherapeutics of dysentery would have to take this into account, and, moreover, should not neglect the relations of bacteriophages to the therapeutic treatment of dysentery and the influence of injected anti-dysenteric sera on these relations.

In my own opinion—which is subject to correction—such action would have to include the following :

I. *Tests of known sera in cases of dysentery of known etiology, under the guidance of reliable experts.*

The tests would have to include the following :

(*a*) Anti-toxic Shiga serum.

(*b*) Serum produced with Shiga bacilli.

(*c*) Sera produced with various mannite fermenting strains.

(*d*) Polyvalent sera.

The manufacture and titration of sera intended for this purpose should be entrusted to a competent serum institute.

II. (Parallel with I.) *Investigations relating to processes of titration.*

(*a*) Test of Olitzky and Kligler's theory.

(*b*) Investigation of the toxins and anti-toxins of mannite fermenting cultures.

(*c*) Suitability of rabbits as compared with white mice for the titration of Shiga toxins : relation between rabbit and mouse toxicity.

(*d*) A study of the toxins and their antitoxin neutralising powers of various Shiga strains.

(*e*) Comparative titrations of dysentery antitoxin in accordance with the methods of :

Schottelius ;
Kraus and Doerr ;
Sachs and Georgi ;
Kolle and Heller

in various institutions, *the same anti-sera and the same toxins being used.*

(*f*) Preliminary investigation regarding the stability of the toxins and of the antitoxin sera kept in dry form.

(*g*) Investigations to determine the best method of measuring the bactericidal properties of sera.

(*h*) Investigations regarding the relation between serotherapeutic and the phenomenon of bacteriophages.

Such a programme would be so comprehensive as to make a division of labour both desirable and reasonable. At the London Conference each expert was left free to attack the question in all its complexity at any point whatever and also to carry on experiments up to any point. This method would perhaps be suitable in the case of very large institutes, but for smaller ones, which are financially dependent on donations from the Committee, it is obvious that the detailed information which they can supply would be limited, and perhaps some aspects of the question would be inadequately dealt with, while others would be treated twice or even oftener. If, as I think probable—the proposed second Conference at Paris should lead to no definite conclusion but should recommend a continuation of the investigations, a carefully defined division of labour would appear to be desirable.

TITRATION OF TOXINS

The animals on which toxin experiments were carried out were rabbits weighing 1,500 gr., and white mice, these animals being susceptible to toxins.

In all cases, except when other methods of introduction are expressly mentioned, the toxin was inoculated into the auricular vein of the rabbit or into the caudal vein of the white mouse. The volume injected was 2 cc. in the case of rabbits, and 0.5 cc. in that of white mice. In order to bring the quantities of toxin introduced up to this amount, a peptone bouillon with a Ph = 7.5 was used.

In all cases the "dysentery toxins" tested were the bouillon culture filtrates of Shiga bacilli. These bouillon cultures were filtered through Reichel filter candles, and were preserved under toluol in refrigerators, in flasks impervious to light. For each experiment a fairly large quantity (12 cc.) of filtrate was withdrawn by means of a sterilised pipette, then drawn through small sterilised paper funnels moistened with bouillon, and finally freed from toluol by being placed for one hour in a thermostat at a temperature of 37° centigrade.

The measurement of the quantity of toxin to be inoculated was carried out by means of accurately graduated pipettes. Special attention was given to quantitative injection, particularly intravenous quantitative injection, since partial subcutaneous

inoculations of toxin solutions exercise a very great influence on the results obtained, *particularly in the case of white mice,* and the series of experiments are rendered quite useless.

The animals were observed for very considerable periods of time in order to note cases of retarded death. Organs of all the animals were examined *post mortem* macroscopically and, when it appeared desirable, microscopically also.

In view of the object of the experiment, the titrations of the toxin solutions were carried out in *parallel experiments* on both species of animals; inoperative, weak, medium and strong filtrates were used in order to ascertain by this means, if possible, quantitative differences as regards the susceptibility of rabbits and mice respectively.

A. Inactive Filtrates

1. *Bouillon culture filtrates.* — The alkalinity of bouillon before inoculation, was such that 6 cc. of normal Na OH added to one liter would produce a neutral reaction using phenolphtalein as an indicator. Age of culture at the time of filtration : 7 days.

(a) *White Mice.*

Body weight	Dose inoculated	Result
16 gr.	0.5 cc.	All mice lived for 14 days without any recognisable symptoms of illness.
13 »	»	
16 »	0.4 »	
14 »	»	
16 »	0.3 »	
17 »	»	
16 »	0.2 »	
14 »	»	
15 »	0.1 »	
17 »	»	
17 »	0.05 »	
13 »	»	

(b) *Rabbits.*

Weight	Rabbit No.	Dose inoculated	Result
1,590 gr.	T 1	2.0 cc.	Remained free from symptons
1,580 »	T 2	1.0 »	
1,500 »	T 3	0.75 »	
1,570 »	T 4	0.5 »	
1,500 »	T 5	0.25 »	

2. *Agar filtrate.* — The growths of strain Dys. A. in two agar-flasks were suspended in 100 cc. 0.85% NaCl solution. The suspension was kept for 24 hours at a temperature of 37° C, and then a further 24 hours at ordinary room temperature. It was then filtered through a Reichel filter candle and the filtrate preserved under toluol. The experiment was carried out six days after filtration.

(a) *White Mice.*

Body weight	Dose inoculated	Result
14 gr.	0.3 cc.	Remained free from symptoms.
12 »	0.2 »	
13 »	0.1 »	
12 »	0.05 »	
13 »	0.01 »	
12 »	0.05 »	

(b) *Rabbits.*

Body weight	Rabbit No.	Dose inoculated	Result
1,720 gr.	T. 69	2.0 cc.	Remained free from symptoms.
1,760 »	T. 70	1.0 »	
1,700 »	T. 71	0.5 »	
1,690 »	T. 72	0.25 »	

3. *Bouillon culture filtrate.* — Alkalinity of the bouillon : the alkalinity of the bouillon before inoculation was such that 8 cc. of n — NaOH added to one liter would produce a neutral reaction using phenolphtalein as indicator. Age of culture at the time of filtration, 14 days. Strain : "Müller Wien". Tested immediately after filtration.

(a) *White Mice.*

Body weight	Dose inoculated	Result
17 gr.	0.5 cc.	Remained free from symptoms.
16 »	0.3 »	
15 »	0.2 »	
16 »	0.1 »	
17 »	0.075 »	
16 »	0.005 »	

(b) *Rabbits.*

Body weight	Rabbit No.	Dose inoculated	Result
2,520 gr.	T. 117	2.0	Remained free from symptoms.
1,270 »	T. 118	1.5	
1,500 »	T. 119	1.0	
1,550 »	T. 120	0.25	

B. Filtrates Weak in Toxin

4. *Bouillon culture filtrate* (Strain : "Kohn Wien"). — Alkalinity of the bouillon before inoculation was such that 6 cc. of n—Na OH added to one liter would produce a neutral reaction using phenolphtalein as indicator. Age of culture before filtration : 16 days. Tested 11 days after filtration.

(a) *White Mice.*

Body weight	Dose inoculated	Result
16 gr.	0.5 cc.	Dead in 3½ days
16 »	0.4 »	» 3½ »
15 »	0.3 »	» 3 »
16 »	0.2 »	» 7½ »
16 »	0.1 »	Remained free from symptoms.
17 »	0.075 »	
16 »	0.05 »	

(b) *Rabbits.*

Body weight	Rabbit No.	Dose inoculated	Result
1,470 gr.	T. 25	2.0 cc.	Dead in 1½ days, moderate ædema of the mucous membrane of the cæcum.
1,410 »	T. 49	1.0 »	Dead in 2 days, p. m. negative.
1,460 »	T. 26	0.5 »	After 3 days marked paresis posterior extremities. After 5 days again free from symptoms and survives.

5. *Bouillon Culture Filtrate* (Strain : "Saloniki"). — Age of bouillon culture before filtration : 12 days. Tested 14 days after filtration.

(a) *White Mice (large).*

Body weight	Dose inoculated	Result
25 gr.	0.2 cc.	Dead in 2½ days.
24 »	0.1 »	Remained free from symptoms.
24 »	0.075 »	
24 »	0.05 »	Dead in 2½ days.
24 »	0.025 »	Remained free from symptoms.
23 »	0.01 »	
23 »	0.0075 »	
22 »	0.005 »	

(b) *White Mice (small).*

Body weight	Dose inoculated	Result
15 gr.	0.2 cc.	Paralysis post. Dead in 3½ days.
16 »	0.1 »	Remained free from symptoms.
15 »	0.075 »	
15 »	0.05 »	
15 »	0.025 »	
15 »	0.01 »	
15 »	0.0075 »	
15 »	0.005 »	

(c) *Rabbits.*

Body weight	Rabbit No.	Dose inoculated	Result
1,500 gr.	T. 169	2.0 cc.	Paresis, dead 4th day.
1,670 »	T. 170	1.0 »	Remained free from sypmtoms.
1,450 »	T. 171	0.5 »	
1,650 »	T. 172	0.25 »	

C. Filtrates of Medium Strength

6. *Bouillon culture filtrate* (Strain : "Dys. A"). — Age of culture before filtration : 12 days. Alkalinity of the bouillon before

inoculation 6 cc. of n—Na OH solution below the neutral point (phenolphtalein as indicator). Tested 4 days after filtration.

(a) *White Mice.*

Body weight	Dose inoculated	Result
15 gr.	0.2 cc.	Dead in 2½ days.
13 »	0.2 »	» 2 »
16 »	0.1 »	» 2 »
17 »	0.1 »	» 2 »
17 »	0.075 »	» 3 ¼ »
16 »	0.75 »	Remained free from symptoms.
20 »	0.05 »	
20 »	0.05 »	
25 »	0.025 »	
22 »	0.025 »	
23 »	0.01 »	
18 »	0.01 »	

(b) *Rabbits.*

Body weight	Rabbit No.	Dose	Result
1,350 gr.	T. 11	2.0 cc.	Dead in 3/4 days, p. m. negative.
1,280 »	T. 12	1.0 »	Dead in 1½ days, p. m. negative.
1,150 »	T. 13	0.5 »	Dead in one day after paralysis, p. m. negative.
1,200 »	T. 14	0.25 »	Remained free from symptoms.
1,200 »	T. 15	0.1 »	

7. *Bouillon culture filtrate* (Strain : "Saloniki"). — Alkalinity of the bouillon : 5 cc. of n—Na OH solution below neutral point (phenolphtalein as indicator). Age of culture : 13 days. Tested 48 hours after filtration.

(a) *White Mice.*

Body weight	Dose inoculated	Result
18 gr.	0.5 cc.	Dead in 3¾ days.
17 »	0.4 »	» 3½ »
17 »	0.4 »	» 3½ »
17 »	0.3 »	» 4 »
15 »	0.2 »	» 4 »
15 »	0.1 »	» 2½ »
15 »	0.1 »	» 9½ »
15 »	0.075 »	Remained free from symptoms.
18 »	0.05 »	
15 »	0.05 »	
18 »	0.025 »	
18 »	0.025 »	
14 »	0.01 »	
14 »	0.01 »	

(b) *Rabbits.*

Body weight	Rabbit No.	Dose	Result
1,590 gr.	T. 201	2.0 cc.	Dead in 3/4 day, p. m. negative.
1,040 »	T. 202	1.0 »	Dead in 3/4 day. Commencing œdema of cæcum.
1,560 »	T. 203	0.75 »	Dead in 1½ days cæcal œdema.

Body weight	Rabbit No.	Dose	Result
1,500 gr.	T. 204	0.5 cc.	Dead in 1½ days; cæcal œdema : hæmorrhage.
1,220 »	T. 205	0.25 »	Paresis anterior limbs. Dead in 1½ days, p. m. negative.
1,570 »	T. 206	0.1 »	Paresis anterior, after 4 days. After 6 days recovered; survived.

8. Repetition after 4 days of the previous experiments with filtrate referred to in (7) in order to determine the *uniformity of the results.*

(a) *White Mice.*

Body weight	Dose inoculated	Result
18 gr.	0.5 cc.	Dead in 2½ days.
17 »	0.4 »	» 4½ »
18 »	0.3 »	» 2 »
17 »	0.2 »	» 3½ »
15 »	0.1 »	Paralysis on 5th day. Dead in 6½ days.
15 »	0.075 »	Dead in 12½ days.
15 »	0.05 »	Remained free from symptoms.
18 »	0.025 »	
14 »	0.01 »	Dead in 8½ days.

(b) *Rabbits.*

Body weight	Rabbit No.	Dose	Result
1,700 gr.	T. 27	2.0 cc.	Dead in 1 day; moderate cæcal œdema; isolated hæmorrhage.
1,710 »	T. 28	1.0 »	Dead in 1¼ days; marked cœcal œdema.
1,770 »	T. 29	0.75 »	Long paralysis; dead in 7½ days; marked cæcal œdema, extensive hæmorrhage, 2 ulcers in the stomach and numerous dark erosions.
1,750 »	T. 30	0.5 »	Dead after ¾ day; marked cæcal œdema.
1,700 »	T. 31	0.25 »	Paralysis after 2 days, lay on its side, died in 3 days; marked cæcal œdema.
1,730 »	T. 32	0.1 »	Paralysis after 2 days, lay on its side, dead in 3½ days; marked cæcal œdema.
1,570 »	T. 33	0.075 »	After 4 days slight paresis; recovered and survived.
1,500 »	T. 34	0.05 »	Free from symptoms.

D. Strong Toxin Solutions

9. *Bouillon culture filtrate* (Strain : "Dys. A"). — Age of the bouillon culture : 12 days. Alkalinity of the bouillon before

inoculation : 6 cc. of n—Na OH below the neutral point (phenolphtalein as indicator). Tested 24 hours after filtration.

(a) *White Mice.*

Body weight	Dose inoculated	Result
14 gr.	0.5 cc.	Dead in 1½ days.
16 »	0.5 »	» 2½ »
13 »	0.4 »	» 2½ »
15 »	0.4 »	» 4 »
15 »	0.3 »	» 2½ »
14 »	0.3 »	After 2½ days marked paralysis of the hind legs lasting up to the 5th day. *Definitely survives.*
14 gr.	0.2 cc.	Dead in 2½ days.
15 »	0.2 »	» 3¾ »
15 »	0.2 »	» 2½ »
15 »	0.1 »	» 3¾ »
16 »	0.1 »	» 3 »
14 »	0.1 »	Remained free from symptoms.
15 »	0.075 »	Dead in 3 days.
13 »	0.05 »	Dead in 4 days
15 »	0.05 »	» 2½ »
16 »	0.05 »	» 3½ »
15 »	0.025 »	» 2½ »
16 »	0.01 »	Remained free from symptoms.

(b) *Comparative experiments on large and small mice five days after filtration.*

Body weight	Dose	Result
24 gr.	0.2 cc.	Death in 3½ days.
27 »	0.2 »	
25 »	0.1 »	Remained free from symptoms.
25 »	0.1 »	
25 »	0.075 »	
26 »	0,075 »	Dead in 2¾ days.
25 »	0.05 »	
23 »	0.05 »	
27 »	0.025 »	Remained free from symptoms
23 »	0.025 »	
22 »	0.015 »	
24 »	0.01 »	

As compared with

15 gr.	0.2 cc.	Dead in 2½ days.
16 »	0.1 »	» 3¼ »
15 »	0.075 »	» 3¼ »
15 »	0.075 »	» 2½ »
16 »	0.05 »	» 2½ »
15 »	0.025 »	» 3½ »
16 »	0.01 »	Remained free from symptoms.

(c) *Rabbits.*

Body weight	Rabbit No.	Dose	Result
1,650 gr.	T. 7	1.0 cc.	Dead in ¾ day, p. m. negative.
1,800 »	T. 8	0.75 »	» ¾ » »
1,500 »	T. 6	0.5 »	» 1 day, »
1,700 »	T. 9	0.25 »	» 1½ days, »
1,550 »	T. 17	0.25 »	» 1½ » »
1,800 »	T. 39	0.25 »	» 2½ » Œdema of the intestine.
1,770 »	T. 10	0.1 »	On the second day paresis of the forelegs, on the 3rd paresis of the hind legs. Animal lay on its side but took abundant food. This condition lasted for 4 days, then the rabbit again recovered the use of its limbs. On the 8th day paralytic relapse, dead on the 9th; commencing œdema of the mucous membrane of the cæcum, hæmorrhages.

10. *Bouillon culture filtrate* (Strain : "Dys. A"). — Age of broth culture : 17 days. Tested 13 days after filtration.

(a) *White Mice.*

Body weight	Dose	Result
18 gr.	0.4 cc.	Dead in 1½ days.
» »	0.2 »	» 3½ »
» »	0.1 »	» 10½ »
» »	0.1 »	» 3 »
» »	0.1 »	» 3 »
» »	0.075 »	» 2½ »
» »	0.05 »	Remained free from symptoms.
» »	0.025 »	
» »	0.01 »	

(b) *Rabbits.*

Body weight	Rabbit No.	Dose	Result
1,700 gr.	T. 50	1.0 cc.	Dead in ¾ day, p. m. negative.
1,680 »	T. 51	0.5 »	» 1 »
1,650 »	T. 52	0.25 »	Paralysis. Dead in 3½ days, marked cæcal œdema, hæmorrhages of the mucous membrane of the appendix, of the wall of bladder and in pericardium.
1,620 »	T. 50	0.1 »	Paresis on 4th day, which quickly subsided; animal survived.

11. *Bouillon culture filtrate* (Strain : "Dys. A"). — Alkalinity of the bouillon before inoculation : 8 cc. n—NaOH solution below the neutral point (phenolphtalein as indicator). Age of the bouillon culture : 22 days. Tested 7 days after filtration.

(a) *White Mice.*

Body weight	Dose	Result
14 gr.	0.1 cc.	Dead in 1½ days.
14 »	0.075 »	Paralysis. Dead in 3 days.
14 »	0.05 »	» » 3 »
14 »	0.025 »	» » 3½ »
14 »	0.025 »	» » 4½ »
14 »	0.01 »	» » 3 »
14 »	0.01 »	Remained free from symptoms.
14 »	0.05 »	
14 »	0.005 »	
13 »	0.0025 »	
13 »	0.0025 »	
13 »	0.001 »	
13 »	0.001 »	

(b) *Rabbits.*

Body weight	Rabbit No.	Dose	Result
2,190 gr.	T. 161	0.5 cc.	Dead in ¾ days, p. m. negative.
2,130 »	T. 160	0.2 »	» ¾ » »
2,160 »	T. 159	0.1 »	Paralysis. Dead in 2½ days.
1,950 »	T. 162	0.1 »	Dead in 2 days, œdema and hyperæmia of the cæcum.
1,300 »	T. 166	0.1 »	Dead in 1 day, p. m. negative.
1,220 »	T. 171	0.075 »	» 1½ days, »
1,180 »	T. 165	0.05 »	» ¾ » »
1,225 »	T. 170	0.05 »	» 3½ » marked cæcal œdema.
1,220 »	T. 169	0.025 »	Remained free from symptoms.
1,300 »	T. 168	0.01 »	

E. Are there cases of individual resistance to infection as a result of which many rabbits entirely fail to react to adequate or large doses of Shiga toxin? (H. Sachs)

12. *Bouillon culture filtrate* (Strain : "Dys. A"). — Age of the culture : 12 days. Minimum lethal dose for rabbits of 1,500 gr. 0.1 cc. intravenous.

Effect of injecting 2 1/2 times the lethal dose :

Rabbit No.	Body weight	Dose	Result
T. 500	1,700 gr.	0.25 cc.	Dead in 1½ days, p. m. negative.
T. 501	1,400 »	0.25 »	Paralysis; lay on its side; dead in 5 days, cæcal œdema with extensive hæmorrhages.
T. 502	1,550 »	0.25 »	Dead in 1½ days, p. m. negative.
T. 503	1,700 »	0.25 »	Paralysis; dead in 2½ days; marked œdema of sub-serosa and sub-mucosa of the cæcum; isolated sub-mucosus hæmorrhages.
T. 504	1,570 »	0.25 »	Paralysis; dead in 3 days; marked cæcal œdema.
T. 505	1,800 »	0.25 »	Dead in 2¼ days, slight œdema of the cæcum.

13. *Bouillon culture filtrate* (Strain : "Dys. A"). — Age of the bouillon culture : 17 days. Minimum lethal dose 0.15 cc.
Effect of injecting seven times the lethal dose :

Rabbit No.	Body weight	Dose	Result
T. 508	2,000 gr.	1.0 cc.	Dead in 1½ days, p. m. negative.
T. 509	1,770 »	» »	» 1¼ » »
T. 510	2,000 »	» »	» 1¼ » » marked cæcal œdema.
T. 511	1,400 »	» »	Dead in ¾ day, p. m. negative.

14. *Bouillon culture filtrate* (Strain : "Saloniki"). — Age of the bouillon culture : 17 days. Minimum lethal dose : 0.3—0.35 cc.
Effect of injecting 5-6 times the lethal dose :

Rabbit No.	Body weight	Dose	Result	
T. 512	1,700 gr.	2.0 cc.	Death in 1½ days	
T. 513	1,650 »	2.0 »	» 1½ »	
T. 514	1,580 »	2.0 »	» 1¾ »	*Post mortem* negative.
T. 515	1,500 »	2.0 »	» 1½ »	
T. 516	1,800 »	2.0 »	» 1½ »	
T. 517	1,470 »	2.0 »	» 1½ »	
T. 518	1,600 »	2.0 »	» 2½ »	
T. 519	1,700 »	2.0 »	» 2 »	
T. 520	1,920 »	2.0 »	» ¾ »	moderate œdema.
T. 521	1,520 »	2.0 »	» 1 »	severe œdema.

F. Influence of the blood vessel selected on the result of the intravascular injection of toxin in rabbits

15. *Bouillon culture filtrate* (Strain : "Dys. A"). — Minimum lethal dose (injecting in the auricular vein) : 0.1 cc. All the rabbits in the group experimented on except T. 531 received *0.25 cc.* but the injections were given in various ways.

Rabbit No.	Body weight	Method of injection	Result
T. 524	1,550 gr.	Right auricular vein.	Dead in 1¼ days, p. m. negative.
T. 525	1,700 »	Half in the right auricular vein, the other half a few seconds later in the left.	Dead in 2½ days ; marked œdema of subserosa and sub-mucosa of the cæcum.
T. 526	1,750 »	Half in the right auricular vein, the rest 2 hours later in the left.	Dead in 3 days, severe cæcal œdema.
T. 527	1,500 »	In the right femoral vein.	Remained permanently free from symptoms till on the 3rd day, transitory slight signs of paralysis in the forelegs.

Rabbit No.	Body weight	Method of injection	Result
T. 528	1,620 gr.	In the central branch of the left carotid.	As T. 527.
T. 529	1,900 »	In the peripheral branch of the left carotid.	Dead in ¾ day, p. m. negative.
T. 530	1,700 »	In the peripheral branch of the left carotid.	Paralysis after 2 days, dead in 2½ days, symptoms of cæcal œdema.
T. 531	1,650 »	0.1 cc. (*i.e.*, the minimum lethal dose) in the peripheral branch of the left carotid.	Dead after 4½ days, marked cæcal œdema.

Conclusions

1. Both rabbits and white mice are susceptible to the toxins contained in bouillon culture filtrates of the Shiga cultures.

2. A filtrate which is non-toxic for rabbits is found equally innocuous for white mice, and *vice versa*. As regards toxic filtrates, there is a considerable parallelism in the response of both types of animal, as is shown by a comparison of the minimum doses ascertained by the titration for a whole series of toxic solutions.

Lethal minimum doses (*intravenous inoculation*) *for rabbits and white mice.* Each pair (*i.e.*, each horizontal line) refers to the same toxin solution.

No. of the toxin solution	Minimum lethal dose	
	(a) for rabbits	b) for white mice
1.	not determinable.	not determinable.
2.	»	»
3.	»	»
4.	2.0 cc.	0.2 cc.
5.	1.0 »	0.2 »
6.	0.5 »	0.1 »
7.	0.25 »	0.075—0.1 cc.
8.	0.1—0.025 cc.	0.075 cc.
9.	0.1 cc.	0.025 »
10.	0.05 »	0.01 »

On the whole, the minimum lethal doses for rabbits (of 1,500 gr.) to those for white mice (of 15 gr.) are in the ratio of 5 : 1; on the basis of the weight of the bodies in grammes the proportion is 1 : 20, *i.e.*, white mice have 20 times more resisting power to the Shiga toxin than rabbits.

3. From the conclusion set forth in paragraph 2 and from the *identity of the symptoms of poisoning* in both species of animal (symmetrical paralysis of the extremities, particularly of hind legs, bleeding diarrhœa) it is evident that it is a *homogeneous toxin*

which affects both rabbits and white mice. It is very improbable that two or more toxic components mixed in variable proportions are present in the toxin solutions (entero-toxin, neuro-toxin, etc.), for it must be assumed that both rabbits and mice are not only susceptible to the same "partial toxins" but that the difference in the susceptibility of each species is the same in respect of each "partial toxin". This view is borne out by the fact ascertained by special experiments (which need not be related here) that, if the toxin solutions are weakened by heat, their toxic property is reduced in proportionally equal degree for rabbits and for white mice.

4. Whether alterations in the *intestines* take place in the case of rabbits after the inoculation of the toxin solution is a question which does not depend, or does not exclusively depend, on the composition of these solutions (*i.e.*, the quantity of "entero-toxin" which they contain and the relation of these hypothetical components to the "neuro-toxin" of Olitsky and Kligler), but upon factors which are governed by the *condition of the animals subjected to the inoculation* at the time of the injection, and which may possess either a constitutional or a conditional character. This is the only explanation for the fact that the *same dose of one and the same toxic-filtrate* produces serious changes of the intestines in one group of rabbits, slight alterations in another group, and no changes at all in a third group (see test No. 11), or that, in two series of tests with the same toxin solution, the changes in the intestines are absent or inconsiderable in one case, while in another case they are found to be extremely marked after each dose (see tests 7 and 8). A further illustration of this phenomenon is given below :

Bouillon culture filtrate (Strain : "Dys. A"). Age of the bouillon culture 17 days; minimum lethal dose : 0.05 cc.

Body weight	Rabbit No.	Dose	Result
1,870 gr.	T. 540	0.4 cc.	Dead 1st day : cæcum œdematous, dark red bloody infiltration of mucosa, the surface as if strewn with fine grey powder (epithelial necrosis). Sub-mucosa bleeding in the upper part of the large intestine.
1,500 »	T. 541	0.4 »	Dead in 1½ days; severe cæcal œdema, *no hæmorrhage.*
2,000 »	T. 542	0.4 »	Dead in 1¼ days; cæcum : severe œdema of sub-serosa and sub-mucosa, hæmorrhagic infiltration and superficial necrosis of the mucosa of the cæcum, bleeding in the large intestine; the cæcum and the large intestine filled with fluid blood.
2,050 »	T. 543	0.4 »	Dead in 3½ days; *no œdema;* infiltration of the mucosa of the cæcum. Necrosis of the epithelium. Cæcum quite empty.

Body weight		Rabbit No.	Dose	Result
2,100 gr.		T. 544	0.4 cc.	Dead in 2 days, severe cæcal œdema; *no hæmorrhage;* no necrosis.
2,350 »	Animals of the same litter	T. 545	0.4 »	Dead in 1 day; p. m. *negative.*
2,100 »		T. 546	0.4 »	Dead in ¾ day, severe cæcal œdema; 2 very small punctiform petechials.
2,390 »		T. 547	0.4 »	Dead in ¾ day; moderate cæcal œdema; no hæmorrhages.
1,500 »		T. 548	0.4 »	Dead in ¾ day; very slight cæcal œdema.
1,670 »		T. 549	0.4 »	Dead in 1 day, distinct but slight cæcal œdema.

5. The selection of the blood vessel into which the Shiga toxin is injected in the case of rabbits has a certain influence on the effect produced; partial neutralisation of the toxin occurs if the toxin is introduced into the veins of the hind legs.

6. The results obtained in white mice may differ considerably if the intravenous injections of the toxin are not made on the principle of quantitative inoculations.

This is also true as regards rabbits, but is less important in the case of these animals, because the resulting technical requirements of the test can very easily be satisfied. In the case of white mice, it is much harder to introduce the whole of the injection into the caudal veins of the tail: (1) because the volume of 0.5 cc. must not be exceeded; (2) because the mouse is particularly susceptible to air embolism; (3) because the introduction of the canule into that vein is much harder, even for specially trained persons, than the insertion into the auricular vein of a rabbit.

7. During the course of experiments related here it was not possible to observe whether, in some cases, individual rabbit or white mouse fail to react to high multiples of the lethal dose of a toxin solution (Sachs).

8. The advantages and disadvantages offered by rabbits and white mice respectively as experimental animals in regard to the testing of dysentery toxin, according to my experience, are as follow :

White mice are cheaper and, in many countries, more easily obtainable than rabbits. The difference in price, per head, is sufficiently large to be worth serious consideration in any agreements as regards international standardisation of methods; the lower cost of mice is partly discounted by :

(*a*) Unsuccessful inoculations, the percentage of which varies according to the skill of the operator;

(*b*) The necessity of using at least *two mice* for each dose;

(*c*) By the fact that the above method does not always lead to clear results in consonance with those already obtained in such cases when, in view of wide divergences of the original dose, it is necessary to carry out subsequent experiments in order to determine the precise values of the interpolate intermediate doses.

It is found preferable, in the end, to carry out the titrations, *a priori*, in such extreme limits and with such gradations that it may be possible to obtain a clear result, affording all the required information. But this method of working involves, as is well-known, the use of a large number of animals.

If the conditions which have just been laid down are adhered to, it is possible even in the case of white mice—by forgoing to some extent the material advantages of smaller experimental animals—to carry out *toxin titrations*, which enable a minimal lethal dose or a certainly lethal dose to be fixed with sufficient *accuracy*, and in such a manner that the *standard obtained* can be *reproduced*. I had, indeed, the impression that rabbits are nevertheless superior to white mice, especially as regards the latter point, *i.e.* in respect of the *constant effect* of certain weak doses of toxin regarded as being "exactly lethal", and in respect of the *constant effect of small multiples* (2 to 4 times) of such exactly lethal quantities, and I therefore urge that the use of rabbits for purposes of testing *dysentery toxins and the corresponding antitoxins should be maintained and white mice should not be substituted for them*, — either as an exclusive method or as a parallel process, of equal value.

I am further strengthened in this view by the following considerations :

(*a*) The greater suitability of rabbits as compared with white mice does not lie exclusively in their fairly considerable and uniform susceptibility to toxin. If combinations of toxin and antitoxin serum, generally a mixture of toxin and antitoxin, are injected the reaction to these combinations is influenced not only by susceptibility to toxin (dissolution of the union between toxin and antitoxin, affinity of the poison to the cells of the body, conditions of elimination of heterologous proteins). As will, however, be shown below, rabbits stand the test better than white mice as far as the titration of mixtures of toxin and serum, in which the quantity of the serum varies while the amount of the toxin remains constant, is concerned.

(*b*) Exact dosage is easy in the case of rabbits and difficult in the case of mice. *Errors in measuring* the doses, and errors in the quantity *absorbed* by the syringes or in the quantity of toxin *injected into the veins* are relatively greater in the case of mice in consequence of the lower (permissible) injection volume. *Errors in injection* are, moreover, immediately visible to the naked eye and are known to the operator in each *individual case* when he is dealing with rabbits, but not when he is dealing with white mice. The explanation of irregularities in the series of tests by errors in injection is therefore as arbitrary as it is tempting when the latter species of animal is used. If various methods are available for measuring a process of nature, the method which involves the fewest possible chances of error (either in number or in degree) is always chosen, unless the accuracy of the results may be regarded as irrelevant to the particular question which is to be solved, a standpoint which cannot *at present* be apopted in determining the value of dysentery sera. The cost of the test is only a mettar

for consideration "ceteris paribus", inasmuch as it exercises but little influence on the price of anti-toxic sera and may be reduced by the centralisation of tests within each State. (The State Sero-Therapeutic Institute at Vienna, the financial situation of which is specially precarious, still uses rabbits for testing its dysentery sera.)

II. DETERMINATION OF THE VALUE OF ANTITOXIC DYSENTERY SERA

The test was carried out by varying the dose of the serum while maintaining unchanged the quantity of the toxin. The latter was always a *known multiple* of the dose *certe letalis* of a particular bouillon culture filtrate.

The neutralisation of the toxin by the antitoxic serum was effected either *in vitro* (method of "mixed injection") or *in vivo* (process of *Kraus* and *Doerr* for ascertaining the therapeutic value).

For the purpose of testing by the method of mixed injection the appropriate quantity of the toxin was measured off and placed in a glass vessel, the serum was added, mixed with the toxin as thoroughly as possible, and the mixture was kept for an hour at a temperature of 37° C. This period of time is sufficient according to the accounts of *Todd* and *Y. Fukuhara* (*Journal of Immunology*, Vol. IV. No. 5, 1919) in all cases; indeed the reports of *Fukuhara*, who adopts it as the standard period, show that equally good results may be obtained after a much shorter contact between the toxin and the serum. These mixtures of toxins and antitoxins were injected :

(*a*) Into the caudal vein of the tail of white mice;

(*b*) Into the auricular vein of rabbits weighing about 1,500 grammes.

Rabbits alone were used when the toxin and antitoxin were injected separately. The importance of the interval between the injection of the toxin and the antitoxin was examined. We injected the dose of toxin into the auricular vein of the right ear and the corresponding amount of serum into the vein of the left ear.

16. Titration of the Serum

The serum was prepared by the State Sero-Therapeutic Institute of Vienna and was designated as Series No. 2407. Its titre was calculated to be equal to 0.2 cubic centimetres when tested by the method of "isolated injection" (toxin and antitoxin separately).

The toxin (bouillon-culture-filtrate) was the one described in Test No. 9. The intra-venous lethal dose was therefore 0.1 cc. for rabbits weighing about 1,500 gr. and 0.025 cc. for white mice of 15 gr.

(*a*) *Result of the "mixed injection" of toxin and antitoxin on rabbits.*

Dose of toxin : 0.25 cc. (2 ½ times the lethal).

Body weight	Rabbit No.	Toxin	Serum	Result
1,700 gr.	A. 40	0.25	+ 0.2 cc.	Remained free from symptoms.
1,450 »	A. 41	»	+ 0.1 »	
1,370 »	A. 42	»	+ 0.075 »	
1,340 »	A. 44	»	+ 0.05 »	
1,350 »	A. 43	»	+ 0.01 »	
1,540 »	A. 45	»	+ 0.005 »	
1,450 »	A. 46	»	+ 0.001 »	
1,600 »	A. 47	»	+ 0.0005 »	On the third day paresis, which subsequently disappeared. Survived.
1,500 »	A. 48	»	+ 0.0001 »	Paralysis on the 2nd day. Dead in 3 days. Noticeable œdema of the cæcum.
1,800 »	A. 49	»	+ (control)	Dead in 2 ¼ days. Moderate œdema of the cæcum.

(*b*) *Result of the "isolated injection" of the toxin and the antitoxin.*

Dose of toxin : 2 ½ times the lethal, *i.e.* 0.25 cc.

(*Kraus* and *Doerr* used for time the lethal dose for ascertaining the so-called "isolated therapeutic value".) The *interval* between the toxin and serum injections is exactly *90 seconds.*

Body weight	Rabbit No.	Toxin	90 Sec. later	Serum	Result
1,830 gr.	A. 34	0.25	»	0.5 cc.	Remained free from symptoms.
1,860 »	A. 35	»	»	0.3 »	
2,000 »	A. 33	»	»	0.2 »	Paralysis on 2nd day; completely paralysed on 3rd; died. Intestinal finding nil.
1,740 »	A. 37	»	»	0.1 »	On 3rd day slight paresis, which rapidly disappeared; survived.
1,780 »	A. 38	»	»	0.05 »	Dead in 3 hrs.: hæmorrhage; infiltration of the lungs. Intestinal findings nil.

Rabbit No. 36 received *first* 0.2 cc. of serum 2407, and after 90 seconds 0.25 toxin and remained free from symptoms.

Discussion : The series of "mixed injection" shows uniform results. — It may be concluded that in this case 0.0005 cc. of serum 2407 neutralised shortly a 2 ½ times the lethal dose of toxin.

The series in the case of the "isolated injection" of the toxin-antitoxin is quite different. The first point to be noted is that, with an interval of 90 seconds, 0.3 cc. of serum were necessary in order to neutralise the same quantity of toxin, whereas this

was neutralised *in vitro* by only 0.0005 cc. This 600-fold increase is an indication that the toxin was absorbed very quickly by the tissues susceptible to toxin, and that therefore the interval between the injection of the toxin and of the serum and the absolute quantity of toxin absorbed *must exercise a decisive influence on the result of the experiments.* The significance of the interval becomes obvious, if we take the rapid absorption of toxin for granted. As regards the absolute quantity of toxin, it must be borne in mind that a quantity sufficient to cause death is more easily absorbed by the susceptible cells within a given time in the case of a larger dose than in that of a smaller one. This is doubtless a function of the concentration of toxin in the blood. If, therefore, the quantity of toxin is doubled or tripled and the *interval between the administration of the toxin and the antitoxin remains constant, this cannot be counter-balanced by a corresponding increase in quantity of antitoxin.* As is shown below, these *a priori* deductions may be verified by experiments. In these circumstances, it is naturally very doubtful whether, in the determination by the method of *"isolated injection"*, we are measuring a property of the sera *which must or may be regarded as a decisive factor for the therapeutic effect of the antitoxic dysentery serum as applied to dysentery patients.*

Apart from this, however, are the results of "isolated injection", as it is shown on corresponding table, very irregular — in fact 0.2 cc. will not neutralise, 0.1 cc. did so. If the first value had been omitted we should have arrived at the conclusion that the serum meets the requirements which Kraus lays down as the minimum of the therapeutic effect, whereas in view of the complete results we should have to decide the rejection of the serum test.

17. Titration of the Serum.

Same serum as in *Experiment No. 16.*
Toxin (Bouillon Culture Filtrate) *somewhat weaker.*
Lethal dose for white mice : 0.075 cc., for rabbits 0.15 cc.

(*a*) *"Mixed injection"* — white mice.

Dose of toxin : 0.2 cc. : 2 ¼ times the lethal dose.

Body weight	Toxin		Serum	Result
15 gr.	0.2 cc.	+	0.004 cc.	Remained free from symptoms.
16 »	»	+	0.002 »	
16 »	»	+	0.0015 »	
17 »	»	+	0.001 »	
16 »	»	+	0.0008 »	
16 »	»	+	0.0006 »	
16 »	»	+	0.0004 »	
16 »		+	0.0002 »	Dead in 2½ days.
17 »	»	+	0.0001 »	» 3½ »
15 »	»	+	0.00005 »	» 4 »
18 »	»	+	(Contr. without serum.)	» 3½ »

(*b*) *"Mixed injection"—Rabbits.*

Dose of Toxin : 1.0 cc. : 6-7 times the lethal dose.

Body weight	Rabbit No.		Toxin		Serum	Result
1,350 gr.	A. 91		1.0 cc.	+	0.01 cc.	Remained free from symptoms.
1,450 »	A. 92		»	+	0.008 »	Paresis of the hind legs on the 3rd day, which rapidly subsided. Survived.
1,470 »	A. 93		»	+	0.006 »	Remained free from symptoms.
1,750 »	A. 94		»	+	0.004 »	Remained free from symptoms.
1,370 »	A. 95		»	+	0.002 »	Paresis on 3rd day, which disappeared. Survived.
1,370 »	A. 96		»	+	0.002 »	Paresis on 3rd day, lay on its side, recovered by the 4th day. Survived.
1,900 »	A. 97		»	+	0.001 »	Dead in 1½ days; intestinal finding—nil.
1,300 »	A. 98		»	+	0.0005 »	Dead in 1½ days; intestinal findings—nil.
1,820 »	A. 104		»	+	0.0004 »	Dead in 1½ days. Severe œdema of the cæcum. Wall of appendix becomes similar to a jelly tube.
1,520 »	A. 105		»	+	0.0002 »	Dead in two days, slight but clear œdema of the cæcum.
1,550 »	A. 106		»	+	0.001 »	Dead in 1½ days, severe œdema, hæmorrhage. Infiltration and superficial necrosis of the mucous membrane.
2,000 »	A. 99	Controls	»	+	—————	Dead in 1½ days. Intestinal findings—nil.
1,770 »	A. 100		»	+	—————	Dead in 1¼ days. Intestinal findings—nil.
2,000 »	A. 101		»	+	—————	Dead in 1¼ days. Moderate œdema of the cæcum.
1,400 »	A. 110		»	+	—————	Dead in 1½ days. Intestinal findings negative.

(*a*) *Isolated injection—Rabbits.*

Toxin dose : 1.0 cc. : 6 to 7 times the lethal dose.

VARIATION OF INTERVAL

(a) *Interval of 90 seconds.*

Body weight	Rabbit No.	Toxin		Serum	Result
2,260 gr.	A. 77	1.0 cc.	90 sec. later	2.0 cc.	Dead in ¾ day, post-mortem negative
2,150 »	A. 61	1.0 »	do.	1.0 »	Dead in 1½ days post-mortem negative.

Body weight	Rabbit No.	Toxin		Serum	Result
2,130 gr.	A. 59	1.0 cc.	90 sec. later	0.8 cc.	Dead in 1½ days, post-mortem negative.
2,100 »	A. 62	1.0 »	do.	0.6 »	Dead in 1½ days, post-mortem negative.
2,090 »	A. 64	1.0 »	do.	0.4 »	Dead in 14 hours, post-mortem negative.
2,050 »	A. 60	1.0 »	do.	0.3 »	Complete paralysis on 2nd day, dead in 3 days.
2,020 »	A. 63	1.0 »	do.	0.2 »	Dead on 2nd day, slight œdema of the cæcum.
		(b) *Interval of 60 seconds.*			
2,200 gr.	A. 78	1.0 cc.	60 sec. later	2.0 cc.	Paralysis. Improved after 4 days. Survived.
2,100 »	A. 79	»	do.	1.0 »	Dead in 1½ days. Post-mortem negative.
2,340 »	A. 80	»	do.	0.6 »	Remained free from symptoms.
1,950 »	A. 81	»	do.	0.4 »	Dead in 1¾ days. Slight œdema of the cæcum.
1,910 »	A. 82	»	do.	0.2 »	Dead in 3 days Post-mortem negative.
		(c) *Interval of 30 seconds.*			
1,850 gr.	A. 83	1.0 cc.	30 sec. later	2.0 cc.	Remained free from symptoms.
1,830 »	A. 84	»	do.	1.0 »	Dead in 2¼ days.
2,370 »	A. 85	»	do.	0.6 »	Dead in 1½ days.
2,190 »	A. 86	»	do.	0.4 »	Remained free from symptoms.
2,200 »	A. 87	»	do.	0.2 »	Dead in 1¾ days. Commencing œdema.
2,220 »	A. 88	»	do.	0.1 »	Dead in 1 day. Post-mortem negative.
		(d) *Interval of 10-20 seconds.*			
2,000 gr.	A. 96	1.0 cc.	20 sec. later	1.0 cc.	Remained free from symptoms.
2,070 »	A. 97	1.0 »	22 do.	0.6 »	Dead in 2 hours (?).
1,900 »	A. 98	1.0 »	12 do.	0.4 »	Remained free from symptoms.
2,090 »	A. 99	1.0 »	12 do.	0.2 »	Remained free from symptoms.
2,070 »	A. 100	1.0 »	15 do.	0.1 »	On the 2nd day paralysis. Dead in 4 days. Injection of mucous membrane of the cæcum.
2,010 »	A. 101	1.0 »	15 do.	0.05 »	On the next day paralysis. Dead in 24 hours.

Discussion. — The titration of the sera by the method of "mixed injection" in the case of mice showed very clearly that 0.2 of the toxin employed was neutralised by 0.0004 cc. of serum 2407.

If—as was already assumed to be probable—the toxin is homogeneous, it was to be expected that 1 c.c. of toxin could just be neutralised in the case of a rabbit by 0.002 cc. of serum 2407, and indeed this was proved to be actually the case. The test, when applied to rabbits, also showed a regular series without any sharp fluctuations.

In the case of titration by the method of "isolated injection" it was ascertained that neither 0.3 cc. nor even multiples of this amount of antitoxin could save the rabbits if the multiple of the lethal dose (test No. 16) was increased from 2 ½ times to 6 times the lethal doses, the interval between toxin and serum injections remaining the same (90 sec.) in both cases. When the interval was shortened, a few rabbits survived free of symptoms, *but not necessarily those which had received the greatest quantities of serum* (see rabbits A. 80 and A. 86). If the interval was reduced to 12 to 15 seconds, the series became more regular (rabbit A. 97 cannot be taken into account) and it was still possible to retard the course of intoxication by the injection at least of 0.2 cc. of serum; indeed even 0.1 cc. at times neutralised the toxin.

But this can only mean that the determination by the method of "isolated injection" may be carried out only under conditions when the saturation of the free toxin in the blood circulation is still possible. It also means that this determination has nothing to do with the "therapeutic value" brought forward by Kraus. Finally there is a remark regarding rabbit A. 78 to be made at this point, which, in a certain sense, falls in the province of the discussion regarding the therapeutic effects of serum. This animal showed signs of severe paralysis on the day after the separate injections of toxin and serum; these symptoms lasted some time, but completely disappeared on the 4th day of the experiment. The rabbit remained alive and did not show signs of cachexia as did the other animals which survived. Is it to be assumed in this case that the serum had a therapeutic effect? Certainly not, for such recoveries are also observed to take place spontaneously in rabbits which have only been injected with toxin (T. 206, T. 33, T. 10, T. 53, T. 552, T. 528), so that the only conclusion to be drawn is that the dose of toxin which took effect was not a lethal dose but only "sub-lethal". The interpretation is different if the serum is only introduced after the symptoms (paralysis) have appeared; but, even when the experiment is carried out in this way, it is necessary to take care that the possibility of *spontaneous recovery* is excluded, in respect of the dose of toxin selected, and also to be certain that no appreciable amount of toxin is circulating in the blood of the animal at the time when the serum is injected. By a "cure" is understood, not the neutralisation of the toxin at the point where it is applied (in the blood), but recovery from the poisoning of the tissues.

18. Titration of the Serum.

Serum : Vienna 2407, as in tests 16 and 17.

Toxin (bouillon culture filtrate; strain "Dys. A"): lethal dose for rabbits 0.05 cc.; for white mice 0.025 cc.

(A) *"Mixed injection"—White Mice.*

Toxin dose : 0.2 cc. : 8 times the lethal dose.

Body weight	Toxin		Serum		Result
16 gr.	0.2 cc.	+	0.003 cc.		Remained free from symptoms.
16 »	0.2 »	+	0.003 »		Dead in 2 days.
14 »	0.2 »	+	0.0025 »		» 4 »
15 »	0.2 »	+	0.002 »		On 5th day paralysis of the hind legs which lasted 8 days and then disappeared. Survived.
14 »	0.2 »	+	0.0015 »		Dead in 3 days.
14 »	0.2 »	+	0.001 »		Dead in 2 days.
15 »	0.2 »	+	0.0005 »		» »
16 »	0.2 »	+	0.00025 »		» »
14 »	0.2 »	+	0.0001 »		» 1½ days.

(B) *"Mixed injection"—White Mice.*

Toxin dose : 0.1 cc. : 4 times the lethal dose.

Body weight	Toxin		Serum		Result
15 gr.	0.1 cc.	+	0.002 cc.		Remained free from symptoms.
16 »	0.1 »	+	0.0015 »		
14 »	0.1 »	+	0.001 »		Dead in 2½ days.
12 »	0.1 »	+	0.0005 »		» 5½ »
13 »	0.1 »	+	0.00025 »		Remained free from symptoms.
12 »	0.1 »	+	0.0001 »		Dead in 2 days.

(C) *"Mixed injection"—Rabbits.*

Toxin dose : 0.4 cc. : 8 times the lethal dose.

Body weight	Rabbit No.	Toxin		Serum		Result
1,370 gr.	B. 17	0.4 cc.	+	0.1 cc.		Remained free from symptoms.
1,350 »	B. 16	0.4 »	+	0.02 »		
1,590 »	B. 15	0.4 »	+	0.005 »		
1,460 »	B. 93	0.4 »	+	0.004 »		On 3rd day paralysis, which disappeared after 48 hours. Survived.
1,700 »	B. 92	0.4 »	+	0.0035 »		Remained free from symptoms.
1,450 »	B. 91	0.4 »	+	0.003 »		
1,700 »	B. 90	0.4 »	+	0.0025		
1,780 »	B. 89	0.4 »	+	0.002 »		Dead on 2nd day. Severe changes in the intestine.
1,340 »	B. 14	0.4 »	+	0.001 »		After 2 days paralysis, dead in 10 days.
1,470 »	B. 23	0.4 »	+	0.00075 »		Dead in ¾ day.

(D) *Isolated Injection—Rabbits.*

(*a*) Toxin dose : 0.4 cc. : 8 times the lethal dose.

Body weight	Rabbit No.	Toxin		Serum		Result
1,960 gr.	B. 58	0.4 cc.	30″ later	2.0 cc.		Remained free from symptoms.
1,900 »	B. 69	0.4 »	» »	1.0 »		Dead in 20 minutes (?).
1,950 »	B. 61	0.4 »	» »	0.6 »		Remained free from symptoms.
1,880 »	B. 62	0.4 »	» »	0.4 »		
1,850 »	B. 63	0.4 »	» »	0.2 »		
1,970 »	B. 64	0.4 »	» »	0.1 »		On the next day incipient paralysis; recovered and survived.

(*b*) Toxin dose : 0.3 cc. : 6 times the lethal dose.

1,820 gr.	B. 65	0.3 cc.	30″ later	2.0 cc.		On the next day very severe paralysis, which, however, slowly disappeared; recovered and survived.
1,800 »	B. 66	0.3 »	» »	1.0 »		Dead in day, p. m. negative.
1,920 »	B. 67	0.3 »	» »	0.6 »		Remained free from symptoms.
1,670 »	B. 68	0.3 »	» »	0.4 »		Dead in 1 day, p. m. negative.
1,850 »	B. 69	0.3 »	» »	0.2 »		Remained free from symptoms.
1,500 »	B. 70	0.3 »	» »	0.1 »		Dead in 1½ days.

(*c*) Toxin dose : 0.15 cc. : 3 times the lethal dose.

1,340 gr.	B. 71	0.15 cc.	30″ later	0.4 cc.		Remained free from symptoms.
1,100 »	B. 72	0.15 »	» »	0.2 »		
1,130 »	B. 73	0.15 »	» »	0.1 »		
1,100 »	B. 74	0.15 »	» »	0.05 »		
1,150 »	B. 75	0.15 »	» »	0.025 »		Dead in one day, p. m. negative.
1,250 »	B. 76	0.15 »	» »	0.01 »		Dead in one day, p. m. negative.

(*d*) Dose of Toxin : 0.15 : Interval 20 seconds.

1,100 gr.	B. 78	20″ later	0.2 cc.		Remained free from symptoms.
1,220 »	B. 79	» »	0.1 »		
1,120 »	B. 80	» »	0.05 »		

Parallel Test to No. 18 D (*a*).

Toxin dose : 0.4 cc. : 8 times the lethal dose.
All other constituents as in the experiment (*a*).

1,500 gr.	D. 1	0.4	Tox.	30″ later	2.0	S.	2407	Remained free from symptoms.
1,400 »	D. 2	0.4	»	» »	1.0	S.	»	
1,430 »	D. 3	0.4	»	» »	1.0	S.	»	Dead in 1 day, slight cæcal œdema.

1,420 gr.	D. 4	0.4	Tox.	30″ later	0.6	S.	2407	Paralysis after 3 days; recovered completely, died, however, suddenly after 14 days.
1,520 »	D. 5	0.4	»	»	0.6	S.	»	Dead in 3½ days.
1,450 »	D. 6	0.4	»	»	0.4	S.	»	On second day paralysis; recovered, *survived.*
1,500 »	D. 7	0.4	»	»	0.2	S.	»	Slight paresis, which disappeared rapidly; *survived.*
1,400 »	D. 8	0.4	»	»	0.2	S.	»	Remained *free from symptoms.*
1,500 »	D. 9	0.4	»	»	0.1	S.	»	Paralysis—dead in 1 day.

Summary of results :

Serum dose
2.0 No effect
1.0 No effect
1.0 Death
0.6 Retarded death
0.6 Death
0.4 Paralysis: recovered
0.2 » »
0.2 No effect
0.1 Death.

Discussion. — The titration by the method of mixed injection in the case of mice produced rather irregular series. It is only the concordance between two tests with differing doses of test-toxin that makes it evident that 0.1 cc. toxin would be neutralised by 0.0015 cc. serum 2407. It might, therefore, have been expected that 0.006 cc. would be the amount necessary for the neutralisation of 0.4 cc. toxin in the case of rabbits; the test showed, however, that 0.0025 cc. are sufficient.

In Tests 16, 17 and 18, the *same serum* was used in each case. Only the *toxin* varied in potency (it was, however, prepared from the same strain "Dys. A" in all cases). If the quantities of serum required for the neutralisation of a single lethal dose of toxin are calculated, the results will be as follows :

No. of the Titration	Mice	Rabbits
16	—	0.0004 cc.
17	0.00016 cc.	0.0003 »
18	0.0004 »	0.0003 »

It is clear that the values ascertained in the case of rabbits are very similar, *while this is not the case* in mice; this can only be ascribed, in the circumstances, to the fact that the accurate determination of a fatal dose is obviously easier in the case of rabbits than of mice.

The results of these series of titration, by the method of isolated injection, are also characterised by *considerable lack of uniformity*

which only disappears as the doses of toxin are diminished and as the intervals between the administration of the toxin and the serum are reduced to 20-30 seconds. But the extreme unreliability of this method becomes evident if a comparison be made between tests No. 16b and 18 D(*c*). In these cases, the test doses are almost equal (2 ½ times the lethal) and the serum is the same; only the intervals differ by 60 seconds; and yet, after the first-named test, the serum was found to be below the limit which is defined by Kraus as reliable, and after the second test it possessed a quite remarkable "therapeutic" power. (See also the parallel titration to 18 D (*a*).)

19. Titration. Serum : Vienna 118

The toxin was the same as in Test 18.

(A) *White Mice.*

Mixed injection. Toxin dose : 0.2 cc. : 8 times the lethal.

14 gr.	0.2	Toxin	+	0.007	Serum 118	(	Remained free from symptoms.	
15 »	0.2	»	+	0.006	» »	)		
15 »	0.2	»	+	0.005	» »		Dead in 3 days.	
15 »	0.2	»	+	0.004	» »		Remained free from symptoms.	
14 »	0.2	»	+	0.0035	» »		Dead in 2 days.	
16 »	0.2	»	+	0.003	» »		Remained free from symptoms.	
13 »	0.2	»	+	0.0025	» »		Dead in	4½ days.
14 »	0.2	»	+	0.0025	» »		»	2½ »
15 »	0.2	»	+	0.002	» »		»	3 »
14 »	0.2	»	+	0.0015	» »		»	2 »
14 »	0.2	»	+	0.001	» »		»	2 »
14 »	0.2	»	+	0.0005	» »		»	2 »
15 »	0.2	»	+	0.00025	» »		»	2 »
14 »	0.2	»	+	0.0001	» »		»	1½ »

(B) *Mixed injection—White Mice.*

Toxin dose : 0.1 cc. : 4 times the lethal.

15 gr.	0.1	Toxin	+	0.002	Serum 115	(	Remained free from symptoms.	
15 »	0.1	»	+	0.001	» »	)		
15 »	0.1	»	+	0.0005	» »		Dead in	5½ days.
15 »	0.1	»	+	0.0004	» »		»	2½ »
15 »	0.1	»	+	0.0003	» »		»	3 »
14 »	0.1	»	+	0.0002	» »		»	4 »
14 »	0.1	»	+	0.0001	» »		»	3 »

(C) *Mixed Injection—Rabbits.*

Dose of Toxin : 0.4 cc. : 8 times the lethal.

Body weight	Rabbit No.	Toxin		Serum	Result
2,000 gr.	B. 21	0.4 cc.	+	0.1 cc.	Remained free from symptoms for 5 days, then died.
1,800 »	B. 20	0.4 »	+	0.02 »	Remained free from symptoms.
1,570 »	B. 98	0.4 »	+	0.01 »	
1,550 »	B. 97	0.4 »	+	0.008 »	
1,220 »	B. 22	0.4 »	+	0.008 »	Paralysis on 3rd day—recovered—survived.
1,550 »	B. 96	0.4 »	+	0.006 »	Remained free from symptoms.
1,850 »	B. 19	0.4 »	+	0.005 »	Paralysis on 3rd day—recovered—survived.
1,580 »	B. 95	0.4 »	+	0.004 »	Dead in 3 days.
1,480 »	B. 94	0.4 »	+	0.002 »	Dead in 1½ days. Cæcal œdema.
1,470 »	B. 18	0.4 »	+	0.001 »	Dead in ¾ day. Cæcal œdema.

(D) *"Isolated injection". Interval 30 Seconds.*

Toxin dose : 0.4 cc. : 8 times the lethal dose.

Body weight	Rabbit No.	Toxin			Serum	Result
2,000 gr.	C. 21	0.4	30 sec.	later	2.0 cc.	Remained free from symptoms.
1,500 »	C. 22	0.4	»	»	1.0 »	
1,500 »	C. 23	0.4	»	»	0.6 »	Dead in ¾ day. Cæcal œdema.
1,550 »	C. 24	0.4	»	»	0.4 »	Dead in ¾ day. Cæcal œdema.
1,400 »	C. 25	0.4	»	»	0.2 »	Dead in 3 days. Cæcal œdema; bleeding.
1,300 »	C. 26	0.4	»	»	0.1 »	Survived free from symptoms.

"Isolated injection". Interval 30 Seconds.

Toxin dose : 0.15 cc. : 3 times the lethal dose.

Body weight	Rabbit No.	Toxin			Serum	Result
1,100 gr.	C. 27	0.15	30 sec.	later	0.4 cc.	Remained free from symptoms.
1,200 »	C. 29	0.15	»	»	0.05 »	
1,100 »	C. 30	0.15	»	»	0.025 »	Dead in ½ day. Hæmorrhage.
1,240 »	C. 31	0.15	»	»	0.01 »	Dead in 1½ days. Cæcal œdema with hæmorrhages.

Discussion. — The results of the tests in white mice show that serum "Vienna 118" may be considered as effective as, or even

more so than, Serum "Vienna 2407" in respect of its antitoxic quality, *when equal doses of the same test-toxin are applied.* The neutralising quantities of serum are as follow :

	Ser. 2407	Ser. 118
For 0.2 cc. of toxin	0.003 cc.	0.003 cc.
» 0.1 » »	0.0013 »	0.001 »

The test when applied to rabbits showed, on the other hand, that the value of "118" *in vitro* was only half as great as that of "2407", or indeed somewhat less (0.006 cc. as compared with 0.0025 cc.).

The series of the titration by the method of "isolated injection" do not call for comment; special attention is, however, drawn to rabbit *C. 26.*

20. Titration. Serum "Gloria Bern", so-called "polyvalent" Serum.

Toxin same as in Tests 18 and 19.

(a) "Mixed injection"—White Mice.

Toxin dose : 0.2 cc. : 8 times the lethal dose.

12 gr.	0.2 Tox.	+ 0.01	S. Gloria	Remained free from symptoms.	
14 »	0.2 »	+ 0.008	»		
10 »	0.2 »	+ 0.006	»		
15 »	0.2 »	+ 0.005	»	Dead in	15 hrs. (?)
15 »	0.2 »	+ 0.004	»	»	6½ days.
16 »	0.2 »	+ 0.035	»	»	2½ »
16 »	0.2 »	+ 0.003	»	»	1 day.
13 »	0.2 »	+ 0.0025	»	»	2 days.
14 »	0.2 »	+ 0.002	»	»	3 »
14 »	0.2 »	+ 0.0015	»	»	3 »
14 »	0.2 »	+ 0.001	»	»	2 »
15 »	0.2 »	+ 0.0005	»	»	2½ »

(b) "Mixed injection"—White Mice.

Toxin dose : 0.1 cc. : 4 times the lethal dose.

14 gr.	0.1 Tox.	+ 0.002	S. Gloria	Remained free from symptoms.	
14 »	0.1 »	+ 0.001	»	Dead in	5 days.
14 »	0.1 »	+ 0.0005	»	»	1½ »
14 »	0.1 »	+ 0.0004	»	»	2½ »
14 »	0.1 »	+ 0.0003	»	»	1½ »
14 »	0.1 »	+ 0.0002	»	»	4 »

(c) "Mixed injection"—Rabbits.

Toxin dose : 0.4 cc. : 8 times the lethal dose.

Body weight	Rabbit No.	Toxin	+ Serum	Result
2,000 gr.	C. 9	0.4 cc.	+ 0.1 cc.	Remained free from symptoms.
1,750 »	C. 8	0.4 »	+ 0.02 »	
1,720 »	C. 7	0.4 »	+ 0.005 »	
1,500 »	C. 99	0.4 »	+ 0.005 »	

Body weight	Rabbit No.	Toxin	Serum	Result
1,610 gr.	C. 100	0.4 cc.	+ 0.004 cc.	Dead in 1½ days, p. m. negative.
1,500 »	C. 25	0.4 »	+ 0.0025 »	Dead in 1½ days. Cæcal œdema.
1,870 »	C. 6	0.4 »	+ 0.001 »	Dead in 1¼ days, marked changes in intestines, œdema, bleeding, necrosis.

21. Titration

Serum W 24. Warsaw.

The same toxin as in the three previous tests.

(a) "Mixed injection"—White Mice.

Was not carried out.

(b) "Mixed injection"—Rabbits.

Toxin dose : 0.4 cc. : 8 times the lethal dose.

Body weight	Rabbit No.	Toxin	Serum "24"	Result
1,400 gr.	C. 4	0.4 cc.	+ 0.1 cc.	Remained free from symptoms.
2,000 »	C. 3	0.4 »	+ 0.02 »	
1,650 »	C. 2	0.4 »	+ 0.005 »	
1,350 »	C. 27	0.4 »	+ 0.0035 »	Paralysis on 4th day, Cachexia on 6th day—recovered, survived.
1,370 »	C. 26	0.4 »	+ 0.0025 »	Dead in 4 days.
2,000 »	C. 1	0.4 »	+ 0.001 »	Dead in ¾ day, slight cæcal œdema.

(c) "Isolated injection"—Rabbits.

Toxin dose : 0.4 cc. : 8 times the lethal dose.

Body weight	Rabbit No.	Toxin	Serum W 24	Result
1,900 gr.	C. 33	0.4 cc. 30 sec. later	0.2 cc.	Dead on 1st day, p. m. negative.
1,990 »	C. 32	0.4 » »	0.1 »	Dead in 2½ days, p. m. negative.
1,330 »	C. 30	0.4 » »	0.05 »	Dead on 3rd day. Severe cæcal œdema with bleeding.
1,310 »	C. 29	0.4 » »	0.025 »	Dead on 1st day, p. m. negative.

(*d*) *"Isolated injection"—Rabbits.*

Toxin dose : 0.15 cc. : 3 times the lethal dose.

Body Weight	Rabbit No.	Toxin		Serum W24	Result
2,500 gr.	C. 37	0.15 cc.	30″ later	0.2 cc.	Remained free from symptoms.
2,300 »	C. 36	0.15 »	»	0.1 »	
1,150 »	C. 35	0.15 »	»	0.05 »	Dead in 2½ days, œdema.
1,000 »	C. 34	0.15 »	»	0.025 »	On 3rd day paralysis, on 6th repeated. Dead on 7th; p. m. negative.

22. Titration

Serum W44, Warsaw.

Toxin : The same as in the four previous tests.

(*a*) *"Mixed injection"—white mice.*

Dose of Toxin is : 0.2 cc. : 8 times the lethal dose.

Body Weight				
9 gr.	0.2 Toxin	+ 0.007	Ser. W44.	Remained free from symptoms.
12 »	0.2 »	+ 0.006	»	Dead in 5½ days.
15 »	0.2 »	+ 0.005	»	Remained free from symptoms.
14 »	0.2 »	+ 0.004	»	Dead in three days.
16 »	0.2 »	+ 0.003	»	Remained free from symptoms.
14 »	0.2 »	+ 0.0025	»	Dead in 2 days.
14 »	0.2 »	+ 0.0025	»	» 1½ »
15 »	0.2 »	+ 0.002	»	» 2 »
14 »	0.2 »	+ 0.0015	»	» 2½ »
14 »	0.2 »	+ 0.001	»	» 1¾ »
14 »	0.2 »	+ 0.0005	»	» 1¼ »

(*b*) *"Mixed injection"—rabbits.*

Dose of Toxin : 0.4 cc. : 8 times the lethal dose.

Body Weight	Rabbit No.	Toxin		Serum W44	Result
2,000 gr.	C. 9	0.4 ccm.	+ 0.1 cc.		Remained free from symptoms.
1,750 »	C. 8	0.4 »	+ 0.02 »		
1,720 »	C. 7	0.4 »	+ 0.005 »		
1,250 »	C. 28	0.4 »	+ 0.0035 »		
1,500 »	C. 25	0.4 »	+ 0.0025 »		Dead in 1½ days, p. m. negative.
1,870 »	C. 6	0.4 »	+ 0.001 »		Dead in 1½ days, moderate cæcal œdema.

(*c*) *"Isolated injection"—rabbits.*

Dose of Toxin : 0.4 cc. : 8 times the lethal dose.

Body Weight	Rabbit No.	Toxin		Ser. W44	Result
2,000 gr.	C. 41	0.4	30" later	0.2 cc.	Dead in 3-4 day, p. m. neg.
1,900 »	C. 40	0.4	»	0.1 »	
2,300 »	C. 47	0.4	»	0.05 »	Dead in 2½ days, p. m. neg.
1,900 »	C. 39	0.4	»	0.05 »	Dead in 1 day, cæcal œdema.
1,750 »	C. 38	0.4	»	0.025 »	Paresis, on the 3rd day some cachexia; dead in 8 days; many partly healed ulcers, covered with slate-grey pigment on the mucous membrane of the cæcum.

(*d*) *"Isolated injection"—rabbits.*

Dose of Toxin : 0.15 cc. : 3 times the lethal dose.

Body Weight	Rabbit No.	Toxin		Ser. W44	Result
2,370 gr.	C. 45	0.15	30" later	0.2 cc.	Remained free from symptoms.
2,300 »	C. 44	0.15	»	0.1 »	On 2nd day paralysis, on 3rd day death.
2,100 »	C. 43	0.15	»	0.05 »	Remained free from symptoms.
2,320 »	C. 42	0.15	»	0.025 »	Dead in 3½ days.

Summary of Results.

As regards the five sera titrated, the quantities which exactly neutralised 0.4 cc. (*i.e.*, 8 times the lethal dose) of a given test-toxin, so that the mixture of toxin and serum, after remaining for one hour at a temperature of 37° C., had no longer an injurious effect upon rabbits, were as follows :

0.0025	cc.	for Serum	2407	(Vienna).
0.0035	»	» »	W44	(Warsaw).
0.005	»	» »	W24	»
0.005	»	» »	Gloria	(Berne).
0.006	»	» »	118	(Vienna).

The differences were well marked and would have been even more evident had higher multiples (10-15 times the lethal dose) been employed, as recommended by Jonmata.

In the titration test in white mice, on the other hand, the above series took the following form :

0.002—0.003 cc.	(pro 0.2 Toxin) for Serum	118.
0.003	» »	2407.
0.003	» »	W44.
0.006	» »	Gloria.

Thus the differences had, in some cases, entirely disappeared and the serial order was changed.

Conclusions.

(1) The titration of antitoxic dysentery sera, by inoculation after preliminary mixing of the toxin and antitoxin *in vitro*, has still to be carried out with *rabbits* (mixture of 10-15 times the lethal dose of toxin with graduated quantities of serum, the mixture to be left standing for one hour at 37° C., and then injected in the auricular vein of the rabbit).

(2) These experiments on *mice* (intravenous injection of the mixture into the caudal vein of the tail) *are not to be recommended.*

(3) Titration by the method of "isolated injection" in rabbits, according to the proposal of Kraus and Doerr, *should not be adopted.* This experiment only furnishes useful and uniform series if the dose of toxin is sufficiently reduced, and if the intervals between the injection of toxin and the subsequent injection of serum are kept constant and sufficiently short (20-30"); but in this case the antitoxin does not have a "therapeutic" effect.

Observations furnish no grounds for believing that sera which are found to be "of high therapeutic value", according to Kraus' definition, produce better results in the treatment of dysentery patients than sera which are highly antitoxic, in the ordinary sense of the word.

(4) Normal Horse Serum does not neutralise Shiga toxins, or not to any appreciable extent.

Experiment.

Toxin, as in Tests 19-22 : 0.4 cc. : 8 times the lethal dose.

0.4 Tox.	+ 1.6 cc.	Normal horse serum.	Dead in 1 day.	Severe cæcal œdema
0.4 »	+ 1.0 »	» »	»	1¼ »	Very severe cæcal and mesenteric œdema.
0.4 »	+ 0.1 »	» »	»	1¾ »	Severe œdema of the cæcum.

STUDY ON THE METHODS OF TITRATION OF ANTI-DYSENTERY SERUM

By Professor Dopter,

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I. Measurement of Antitoxic Power

The Committee decided that it was advisable to titrate the antitoxic and anti-endotoxic potencies of the anti-dysentery serum. This opinion was based upon recent experiments which showed that, in cultures of dysentery bacilli in a liquid medium, it was possible to demonstrate the presence of an exotoxin which could be isolated in bouillon cultures after 5 days, and an endotoxin which appeared in cultures 20 days old, mixed with the exotoxin.

The endotoxin, when injected into rabbits, produces only intestinal symptoms, without paralytic phenomena; the latter arise only when exotoxin are acting.

The experiments undertaken to verify this view were not successful. However, earlier investigations show that the autolysate obtained from cultures 24 hours old on a solid medium could determine the two classes of phenomena: the same results are obtained by injecting dead cultures of the same age.

Moreover, a serum obtained by inoculation with a filtrate of bouillon culture 5 days old neutralises the endotoxin where the latter is injected alone or together with dead microbes.

The existence of an exotoxin is therefore problematical; and consequently we need only determine the antitoxic potency of the serum.

Experiments in the titration of the antitoxic potency were carried out on rabbits, mice, and guinea-pigs. Guinea-pigs had to be discarded, owing to their insensitiveness to the action of the toxin.

Experiments on rabbits had to be abandoned owing to the inequality between the reactions to toxins observed in different individuals.

Mice are undoubtedly the most satisfactory animals. Injections of the mixture of toxin and serum were carried out, for purposes of comparison, subcutaneously and intravenously. The sera used were obtained from horses which had been immunised either with live or dead cultures or with the toxin obtained from cultures in bouillon of 5 and 21 days.

The results were as follows :

1. Whatever serum and toxin were used, the neutralisation results were substantially the same, whether the subcutaneous or

the intravenous method was used. Either may therefore be used indifferently.

2. The best results were derived with serum obtained from horses which had been inoculated only *subcutaneously* with toxin from cultures in bouillon left in the incubator for a week at 37 degrees (J. Dumas).

The results were not so good when the injections into the horse were carried out first subcutaneously and then intravenously; the same applied when the horse received only live cultures, subcutaneously and then intravenously.

2. Measurement of Anti-infectious Potency

Tests are still in progress.

INVESTIGATIONS REGARDING DYSENTERY TOXIN AND ANTITOXIN

From the Cracow Branch of the State Epidemiological Institute at Warsaw

(*Director: Professor Nowak*)

By Dr. Marjan Gieszczykiewicz

University Lecturer in charge of the Serotherapeutic Department,

And Dr. Witold Lipinski,

Bacteriologist at the Institute.

According to the researches of Olitsky and Kligler (*Journal of Experiment. Medic.*, XXXI, No. 1. Jan. 1920), the dysentery toxin which is produced by the dysentery bacilli of the Shiga-Kruse type consists of two constituents—the neurotoxin or exotoxin and the endotoxin or enterotoxin. Each constituent has a special affinity for the organs of the rabbit, and consequently produces effects which are anatomopathologically different.

The above-mentioned authors indicate methods which, in their opinion, are able to separate the neurotoxin from the enterotoxin. In the first of these methods, dysentery cultures of different ages are discussed. Fresh cultures for the most part produce neurotoxin, while older cultures produce enterotoxin. By the second method the two toxin constituents are separated by the application of a higher temperature. The exotoxin is thermolabile; it is rendered inactive by being heated for one hour up to a temperature of 75° C., while the endotoxin is able to resist this temperature. The third method is that in which the various dysentery toxin constituents are neutralised by specific dysentery antitoxin. Olitsky and Kligler maintain that, by inoculating a rabbit with toxins which possess a special affinity for the nervous system, they succeeded in obtaining a serum with an antineurotoxic action. This serum neutralises the neurotoxin, while the enterotoxin remains active.

As a result, the animal dies, showing symptoms of intestinal lesions. It is clear that this method can only be employed after the two dysentery toxin constituents have already been isolated by another method. Otherwise it is impossible to obtain strictly specific antineurotoxic serum and antienterotoxic serum respectively.

Before entering upon our investigations with regard to dysentery toxin, we prepared the culture medium in accordance with the directions given by Olitsky and Kligler in the following manner:

A quantity of beef free from fat was grated and double the quantity of water was poured over it. The following were then added: 1 ½ % peptone, ¼ % of common salt, 1 ½ per thousand of soda

(sod. carb. anhydr. Merck) (calculated in proportion to the quantity of water), and 10 % of albumen solution in the ratio of 1 to 3. The liquid was brought up to Ph. 7.6—7.7 and afterwards sterilised for half an hour at a temperature of 120° C. Each of the flasks contained half a litre of liquid. In order to discover whether, and if so, how far, the ovalbumen is of any importance, a second culture medium was prepared at the same time in precisely the same manner except for the fact that it contained no ovalbumen. The culture mediums were inoculated with dysentery bacilli of the Shiga-Kruse type (strains : Warsaw and 1278). Both dysentery strains had already been proved to be good toxin producers.

In our experiments toxin filtrates (Chamberland or Berkefeld filter) were injected into the auricular vein of the rabbit.

According to the observations of Olitsky and Kligler, neurotoxin appears in bouillon cultures as early as the second or third day. The neurotoxin, the characteristic of which is its affinity for the nervous system of the rabbit, produces symptoms of paralysis, extravasations in the grey matter of the spinal cord, necrotic foci, caryolysis, granular degeneration of the cells, and caryorrhexis. Infiltrations of round cells appear round the blood-vessels, with œdema of the white and grey matter of the spinal cord. Olitsky and Kligler found the greatest changes in the medulla oblongata and in the spinal cord of the rabbit.

Our experiments, the object of which was to isolate the neurotoxin from the enterotoxin, were unsuccessful, that is to say they did not fully confirm the observations of Olitsky and Kligler. Even in five-days-old filtrates we found enterotoxin as well as neurotoxin. Simultaneously with paresis and paralysis of the extremities we observed, almost regularly, diarrhœa of varying intensity, frequently accompanied by violent tenesmus (cf. Tables Nos. 548, 549, 555-574). Though the American authors have also observed diarrhœa, they state that the fæces were not of a pathological nature. The exotoxin should produce no symptoms of inflammation in the intestine. In all the cases which were submitted to the section we found pathological changes, often of great intensity, in the large intestine, dullness of the peritoneum, the blood-vessels distended and engorged with blood, a serous exudate in the abdominal cavity, thickening of the walls of the large intestine, disintegration and swelling of the mucous membrane, dilation of the intestinal blood-vessels, effusions of blood and ulceration in the mucous membrane of the intestine. In one case (Table 548), extensive diphtheritic intestinal ulcers, with peritoneal adhesions, were observed. The death of this animal occurred 24 hours after the intravenous injection of 1 c.c. of bouillon culture filtrate 4 days old. In this case violent and stubborn diarrhœa was also present during three days.

In addition to the intestinal symptoms, we also observed in our experiments paresis and paralysis of the extremities (Tables 556, 557 and 572), as did Olitsky and Kligler.

Our researches regarding the potency of the dysentery toxin

have shown that the dysentery toxin is subject to considerable variations. The strongest toxin was produced by bouillon cultures 5 days old. The toxicity of older cultures diminished in the first days, but increased later, and reached its maximum about the end of the third week.

In connection with the isolation of neurotoxin from enterotoxin by higher temperatures, the following facts were ascertained :

After an hour's exposure to a temperature of 70° C. the dysentery toxin produced symptoms of paralysis. There was, therefore, no destruction of the neurotoxin, as asserted by Olitsky and Kligler (Table 570). A temperature of 75° C. completely destroyed the toxin. In these cases we could record no effects even when the dose injected was many times the volume of the lethal dose (Tables 805, 806, 807, 827, 828). We obtained similar results after heating the dysentery toxin to 80° C. for an hour. Our results confirm the observations of Doerr, who found that after being heated to 80° C. for three minutes the dysentery toxin was completely destroyed. According to Doerr (1), the dysentery toxin was destroyed in one minute by a temperature of 90-100° C., and was weakened in 15 minutes by a temperature of 70° C.

We did not succeed in isolating the toxin constituents by the above-mentioned method; we did not obtain specifically antineurotoxic serum, and consequently experiments of this nature on the lines indicated by Olitsky and Kligler could, *sensu strictiore,* not be repeated. In estimating the value of the dysentery antitoxins of many sera, particularly of weak sera, we observed that the dysentery antitoxin, though not specifically antineurotoxic, rendered the rabbit immune from symptoms of paralysis. In the case in question the animal died showing intestinal symptoms.

It seems that the horse produced more antineurotoxin than antienterotoxin, although it was not treated exclusively with neurotoxin, but with both toxic constituents and with an intravenous injection of live dysentery bacilli.

The addition of albumen to the culture medium was of no particular importance.

The preservation of the dysentery toxin presented great difficulties. The sterilised bouillon filtrates were preserved by the addition of toluol (Merck), as a result of which we noticed a considerable weakening of the dysentery toxin. This led to the formation of slightly flocculent precipitates. In the course of some days the toxicity of the filtrate diminished and at the same time the turbidity and precipitation increased. No similar symptoms were observed when sterilised filtrates were used without the addition of toluol.

In carrying out parallel experiments to estimate the value of the antitoxin, we took account of the Rosenthal method (value test on rabbits) and the Kolle Sachs and Georgi (2) method (value

(1) See Kraus and Levaditi " Technik und Methodik d. Immunitätsforschung ". Vol. 1.
(2) Kolle, Keller, da Mestral. Deutsche med. Woch. 1908, No. 19. Sachs Georgi. Med. Klinik: 1918. No. 25. p. 610.

test of serum on white mice). In testing the value of serum on rabbits the States Serum Control Institute in Warsaw considers that 0.003 cc. of dysentery serum at full strength should neutralise ten times the lethal dose of dysentery toxin. The dose of toxin and the volume of antitoxin are calculated for 1 kilog. body weight.

The dysentery serum was tested in the following manner : the lethal dose of our toxin amounts to, say, 0.03 cc. per kilog. of body weight. Ten lethal doses of the toxin are mixed with a proportionate quantity of serum (0.333 serum solution at 1 : 100). To avoid error double quantities are prepared. The mixture of toxin and antitoxin (1.266 cc.) is brought up to 2 cc. by the admixture of salt solution and is placed in the incubator for half an hour. 1 cc. of the mixture is injected into the auricular vein of the rabbit.

The Institute of Experimental Pathology at Frankfort uses white mice to test the value of the dysentery serum. It finds that 0.001 cc. of the dysentery serum neutralises from 4 to 6 times the lethal dose of toxin. After being kept for half an hour in the incubator 0.5 cc. of the toxin-antitoxin mixture is injected intravenously into white mice.

The advantage of the Rosenthal method rests on the high susceptibility of the rabbit to dysentery toxin. The mixture of toxin and antitoxin, which produces no effect upon the mouse, occasionally proves in a corresponding dose fatal to the rabbit. In addition, the intravenous injections can be carried out much more easily in the case of rabbits.

The varying susceptibility of rabbits introduces a disturbing factor into the method. If a mixture of weak serum with a corresponding dose of dysentery toxin is injected into a number of rabbits simultaneously, it is possible that three may die out of five. This result depends to some extent on the breed of the rabbit, but to an even greater extent on its weight. Rabbits weighing less than 1,000 grammes are more sensitive; those over 1,000 grammes less so. The degree of susceptibility is not strictly in proportion to weight of body. The following observations may be given as an example : 0.02 cc. of dysentery toxin No. 15, per kilo of the weight of the animal, produces, after an incubation of from 24 to 36 hours, typical paralysis of the extremities, diarrhœa, and finally death on the fourth day. A post-mortem examination reveals diphtheritic ulcers of the large intestine. Simultaneous intravenous injections of 0.05 cc. and 0.1 cc. merely produce temporary paresis of the extremities and slight intestinal catarrh respectively; both the animals survive. The varying reaction of rabbits to dysentery toxin constitutes the main difficulty in determining the value of curative dysentery sera. It is highly desirable, therefore, that such experiments should be carried out with animals of the same weight and breed.

A further disadvantage of the Rosenthal method is connected with the special susceptibility of the rabbit to other infections. The material which we have derived from post-mortem examina-

tions reveals pathological changes which in many cases cannot be reconciled with the action of the dysentery toxin. For example, coccidiosis, which alone could not cause death in individual cases, undoubtedly lowered the power of resistance to the introduction of heterogenous albumen and peptone. A comparatively large number of rabbits died without showing the characteristic symptoms for the action of the toxin. The death of these animals was of no use for testing the value of the serum.

We also conducted experiments according to a modified Rosenthal method by using 100 lethal doses instead of 10 lethal doses as is done in testing the value of curative diphtheria serum. We were not successful with this method for the following reasons : (1) In 10 per cent of the cases death occurred immediately, accompanied by anaphylactic symptoms. Death would appear to be due to the action of the peptone (or to other factors independent of dysentery toxin), of which 10 times the usual volume is injected when using the 100-dose method; (2) in 25 per cent of the cases death occurred after 24 hours without clinical symptoms. The negative results of post-mortem examination could not be used as a test for toxic action. In employing the 100-dose method we lose 35 per cent of the animals experimented upon without obtaining useful results.

The Kolle Sachs and Georgi method has none of these disadvantages. Differences of breed and comparatively small differences in the weight of the white mice are matters which are of no importance. The white mouse is less sensitive to dysentery toxin, and, consequently, more constant results are obtained when testing the value of sera. Their lower purchase price and cost of maintenance constitute a further advantage.

Tables

Prot. No.	Date of inoculation	Animal	Quantity and kind of injected toxin	Clinical symptoms	Death	Post-mortem Findings
548	9/VI	Rabbit, weight : 1,050 gr.	1 cc. dysentery toxin filtrated No. 17, 4 days old.	Diarrhœa 10/VI	12/VI	Diphtheritic alterations of colon. Adherences of peritoneum.
549	»	Rabbit, weight : 1,020 gr.	1 cc. dys. tox. filtrated No. 17, 5 days old	Diarrhœa 13/VI	24/VI	Catarrhal alterations of colon.
554	10/VI	Rabbit, weight : 1,000 gr.	1 cc. dys. tox. filtrated No. 17, 6 days old, without albumen	Diarrhœa 10/VI	10/VI	Negative.
555	»	»	1 cc. dys. tox. filtrated No. 17, 6 days old, with albumen	Diarrhœa 11/VI	12/VI	Slight inflammation of colon.
556	12/VI	»	1 cc. dys. tox. filtrated No. 17, 5 days old, with albumen.	Fore-limbs paralysed 13/VI	14/VI	Negative.

Prot. No.	Date of inoculation	Animal	Quantity and kind of injected toxin	Clinical symptoms	Death	Post-mortem Findings
557	»	»	0.5 cc. dys. tox. filtrated No. 17, 6 days old, with albumen	Diarrhœa paralysis 13/VI	14/VI	Negative.
558	»	»	» » 6 days old, without albumen	Diarrhœa 13/VI	13/VI	Inflammation of colon.
571	14/VI	Rabbit, weight : 1,100 gr.	0.1 cc. dys. tox. No. 17 6 days old, without albumen		15/VI	Negative.
572	»	» »	» » 6 days old, with albumen	Paralysis 17/VI	Survived	
573	»	Rabbit, weight : 1,000 gr.	» » 9 days old, without albumen		Survived	
574	»	Rabbit, weight : 1,150 gr.	» » 9 days old, with albumen		Survived	
628	22/VI	Rabbit, weight : 1,300 gr.	» » 14 days old, without albumen	Paralysis 25/VI	Survived	
570	14/VI	Rabbit, weight : 1,200 gr.	0.1 cc. dys. tox. No. 15 Sh. War. Heated to 70° C. for 1 hour	Paralysis 16/VI	17/VI	Negative.
611	21/VI	» »	» » Heated to 80° C. for 1 hour	No symptoms		
638	26/VI	Rabbit, weight : 1,150 gr.	» » Heated to 80° C. for 1 hour	No symptoms		
656	1/VII	Rabbit, weight : 1,050 gr.	0.02 cc. dys. tox. No. 15 Sh. War.	Diarrhœa, paralysis 3/VII	5/VII	
657	»	Rabbit, weight : 1,150 gr.	0.05 cc. dys. tox. No. 15 Sh. War.		Survived	
658	1/VII	Rabbit, weight : 1,150 gr.	0.1 cc. dys. tox. No. 15 Sh. War.		Survived	
635	26/VI	Rabbit, weight : 1,050 gr.	» »	Diarrhœa 28/VI	2/VII	
636	»	Rabbit, weight : 1,000 gr.	0.05 cc. dys. tox. No. 15 Sh. War.		2/VII	

Test of Potency of Anti-dysentery Serum by 100-D. L. Method.

620	21/VI	Rabbit, weight : 1,200 gr.	100 D. L. dys. tox. No. 11 (D. L. 0.02 cc.) + Serum 22 v. 1/IV.		22/VI	Negative.

Prot. No.	Date of inoculation	Animal	Quantity and kind of injected toxin	Clinical symptoms	Death	Post-mortem Findings
637	26/VI	Rabbit, weight : 900 gr.	0.02 cc. dys. tox. No.15. Sh.War.	27/VI Paralysis	28/VI	
621	21/VI	Rabbit, weight : 800 gr.	100 D. L. dys. tox. No. 11 (D. L. 0.02 cc.) + Serum 45+24.	24/VI Paralysis	25/VI	Negative
622	21/VI	Rabbit, weight : 1,250 gr.	100 D. L. dys. tox. No. 11+Serum 22.2/III	22/VI Paralysis	23/VI	Negative.
623	»	Rabbit, weight : 1,300 gr.	100 D. L. +Serum 37 v. 1/II	Anaphylactic shock some minutes after injection.	21/VI	
624	»	Rabbit, weight : 950 gr.	100 D. L.+Serum 45 1/IV			
625	»	Rabbit, weight : 1,100 gr.	100 D. L.+Serum 37 3/IV		24/VI	Negative.
626	»	Rabbit, weight : 1,150 gr.	100 D. L.+Serum 45 2/III		23/VI	»

TEST OF POTENCY OF ANTI-DYSENTERY SERUM ON WHITE MICE, ACCORDING TO KOLLE

Anti-Dysentery Serum No. 24.

542	7/VI	White mouse, weight : 15 gr.	5 doses D. L. dys. tox. No. 11 (D. L. 0.01 cc.) + Serum Kolle 1 (Dilut. 1 : 125)	
543	»	» »	Kolle II (Dilut. 1 : 250)	
544	»	» »	Kolle III (Dilut. 1 : 500)	
575	17/VI	» »	Kolle IV (Dilut. 1 : 1,000)	21/VI
576	»	» »	Kolle V (Dilut. 1 : 2,000)	»
577	»	» »	Kolle VI (Dilut. 1 : 4,000)	20/VI
578	»	» »	Kolle VII (Dilut. 1 : 8,000)	»

Serum 47 = 1.

545	7/VI	White mouse, weight : 15 gr.	5 D. L. dys. tox. No. 11 (D. L. 0.01 cc.) + Serum Kolle I	
546	»	» »	Kolle II	
547	»	» »	Kolle III	
579	17/VI	» »	Kolle V	
580	»	» »	Kolle VI	
581	»	» »	Kolle VII	20/VI

Test of Potency of Anti-dysentery Toxin No. 15 Sh. War.

Prot. No.	Date of inoculation	Animal	Quantity and kind of injected toxin	Clinical symptoms	Death	Post-mortem Findings
686	18/VII	White mouse, weight : 15 gr.	0.005 cc. dys. tox.			
687	»	» »	0.01 cc. dys. tox.		20/VII	
688	»	» »	0.02 cc. »		»	

Anti-dysentery Serum 4 3/IV.

602	20/VI	White mouse, weight : 15 gr.	5 doses D. L. dys. tox. No. 11 (D. L. 0.01 cc.) + Serum 4 Kolle II			
603	»	» »	Kolle III	23/VI Paralysis	23/VI	
604	»	» »	Kolle IV	»	24/VI	
605	»	» »	Kolle V	»	25/VI	
606	»	Control mouse			24/VI	

Serum 1 I/IV.

592	20/VI	White mouse, weight : 15 gr.	Kolle IV			
593	»	» »	Kolle V		23/VI	
594	»	» »	Kolle VI		25/VI	

Test of Potency of Filtrated Dysentery Toxin No. 16.

673	13/VII	White mouse, weight: 15 gr.	0.01 cc.			
674	»	» »	0.03 cc.		16/VII	
675	»	» »	0.05 cc.		»	
680	17/VII	» »	0.02 cc. (D. L. 0.02 cc.)		20/VII	

Test of Potency of Dysentery Toxin No. 15.

772	15/IX	White rabbit, weight : 1,150 gr.	0.025 cc. pro kg	18/IX Paralysis		
773	»	» »	0.03 cc. »	17/IX Paralysis Diarrhœa	18/IX	Diphtheritic alterations of colon.
774	15/IX	Grey rabbit, weight: 1.100 gr.	0.035 cc. pro kg	18/IX Paralysis	Survived	
775	»	Black rabbit, weight: 1.250 gr.	0.04 cc. »	18/IX Paralysis	19/IX	
776	»	» »	0.05 cc. »		18/IX	

Test of Potency of Anti-dysentery Serum 51 Ser. II.

Prot. No.	Date of inoculation	Animal	Quantity and kind of injected toxin	Clinical symptoms	Death	Post-mortem Findings
782	19/IX	White rabbit : 1,200 gr.	10 D. L. + Serum			
783	»	Grey rabbit : 820 gr.	» »	22/IX Paralysis, diarrhœa	23/IX	
784	»	White rabbit : 820 gr.	» »	»	22/IX	
785	»	Grey rabbit : 770 gr.	» »		20/IX	Negative.
786	19/IX	Black rabbit : 1,250 gr.	10 D. L. + Serum	Slight paral.		

No. 15 Dysentery Toxin heated to 75° C. for one hour

805	20/IX	White rabbit : 950 gr.	3. D. L.	No symptoms
806	26/IX	White rabbit : 1,050 gr.	5. D. L.	»
807	»	White rabbit : 900 gr.	2. D. L.	»
827	1/X	White rabbit : 1,500 gr.	20 D. L.	»
828	»	White rabbit : 1,250 gr.	10 D. L.	»

FIRST REPORT ON THE STANDARDISATION OF DYSENTERY SERA

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In order to be able to determine the antitoxic properties of dysentery sera, we require information as to which constituent element of the toxin is of importance in the pathology of dysentery. The sera used for human beings must neutralise this constituent. Therefore, before approaching the question of standardisation, we wanted first of all to gain further knowledge with regard to the formation of toxins and the nature of dysentery intoxication by means of experiments on animals.

Investigations were made in three directions. First, the most suitable conditions for toxin formation had to be ascertained. Secondly, the pathogenic properties of the toxins of the bacterial extracts and of the living bacteria were tested with a view to dividing the toxin if possible into its constituent parts. Thirdly, sera were obtained from horses by immunising with filtrates of young bouillon cultures—"pure toxins"—and of old bouillon cultures, and with living bacteria, in order to discover which element of the bacteria should be used as antigens, and to determine by means of these experiments whether sera which, after their formation, should contain more antitoxins or anti-endotoxins, show different results when standardised.

I. The Dependence of Toxin Formation on the Alkalescence of the Nutrient Medium.

(a) *Modifications in the alkalescence of the nutrient medium under the influence of Shiga-Kruse bacilli.*

The well-known investigations of Doerr have already proved the dependence of toxin formation on the alkalescence of the nutrient medium. In accordance with the state of science in those days Doerr's investigations were carried out without any accurate examination of the free H-Ions content. Later works contain accounts which are to some extent contradictory. In order to examine these conditions more closely, we have investigated the toxicity of bouillon cultures with different Ph contents, having regard not only to the Ph content of the nutrient medium employed, but also to the concentration of free H-Ions in the culture at the moment of examination.

The nutrient medium was prepared in the following way : 200 grammes of water was poured over 1 kilogramme of horse-flesh, which was then boiled and pressed out in a hand-press. This meat extract was diluted to a fifth of its strength, combined with 1% peptone and 0.5% of NaCl, and brought up to Ph 7. In most experiments some yeast was added and left at 37° C. to ferment for 6 hours. The bouillon was then brought to the boil, filtered and brought by the addition of hydrochloric acid or a solution of caustic soda to the desired Ph content. Where no mention to the contrary is made in the reports, 250 cc. of the bouillon was poured into a 1-litre Roux flask and then sterilized in the autoclave for 45 minutes at 100° and for a further 15 minutes at 115°. Sterilization modifies the reaction, namely, the alkaline nutrient media become more acid while the acid media become more alkaline, though the difference never exceeds Ph content 0.4. The bottles are then inoculated with 0.5—1 cc. of a 24-hour Shiga bouillon culture and again tested for their Ph content. The temperature of incubation is 35-36° and the flasks are laid so that the depth of the nutrient medium is only 1 cm. The flasks were provided with a glass tube passing through the cork through which, by means of a sterilized india-rubber tube, the test specimens were extracted. Five strains were examined.

We obtained the growth of the bacteria within Ph 6.5—8.5. In the case of Ph 6.3 and 9.0 we did not observe any growth. (Jonesco, Mihaesti and Popesco, *C. R. Société de Biologie,* 1922, 86, 893, obtained growth within wider limits.)

Under the influence of the bacteria, the Ph content of the culture is modified. We can distinguish here between two periods. In the first, which is of varying length according to the depth of the bouillon culture, the alkaline nutrient media become more acid, while in the case of a certain "neutral" Ph the nutrient media remain unchanged. This "neutral" point is 6.8 for dysentery bacilli. Very alkaline and very acid nutrient media, in which no growth takes place, do not change their Ph contents. After this first period of acidification there begins a second of increasing alkalification during which the Ph content, in the case both of acid "neutral" and alkaline tests, seeks to reach a common maximum which is about 8.5. After a certain time, all bouillon cultures of Shiga bacilli show the same alkaline reaction. The rate at which the alkalescence increases is, probably, dependent on the intensity of the metabolism. The fact which is of practical importance is that it can easily be modified by altering the depth of the bouillon. Table 1 gives details of an experiment of that kind, in which different quantities of the same bouillon were poured into three different bottles and the Ph content was determined after periods of varying length.

Table I.

	Immediately after inoculation	After 3 days	After 5 days	After 7 days	After 11 days	After 16 days
	—	—	—	—	—	—
Depth of the nutrient medium, 1.0	7.6	7.0	7.4	8.1	8.5	8.6
Depth of the nutrient medium, 4.0	7.7	7.2	7.3	7.6	8.0	8.4
With no admission of air (1) . . .	7.8	7.2	7.4	7.4	7.7	7.7

The table shows that, if the depth of the nutrient medium is 4 cm., alkalescence is developed to the extent of more than PH 8 after 11-16 days, whereas if the bouillon layer is not so deep, its presence is observed after 7 days. With no admission of air the PH content remains almost unchanged and after 16 days the bouillon is not more alkaline than immediately after inoculation.

(b) *The dependence of the toxicity of the bouillon on the PH content of the nutrient medium.*

In conjunction with the testing of alkalescence, tests were also carried out to discover the toxicity of the filtrates. As an experiment, 50 cc. was taken away on several occasions, sterilised by the addition of carbolic acid (0.5%) and after 24 hours the bacteria were removed by centrifugalisation and injected into rabbits after being tested as regards sterility. Strain No. 6 was not sufficiently toxic and we therefore give the results of the experiments with strains 1036, 12, 25 and 17.

Table II (*abridged*).

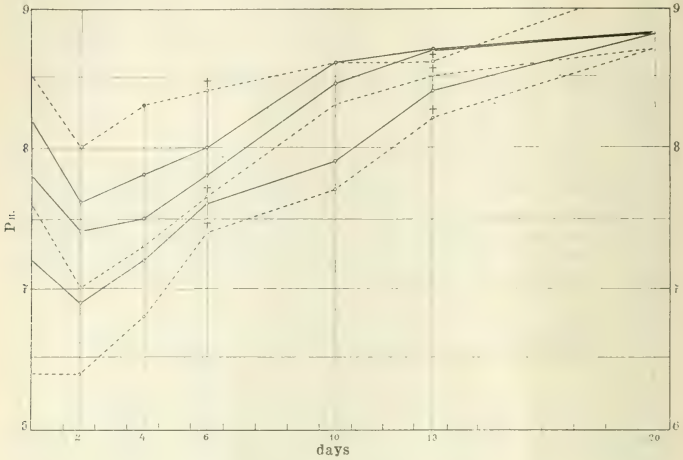
The depth of the nutrient medium was 4 cm. The original PH content was 6, 4; 7, 5; 8, 4. In carrying out the experiment 0.18 cc. was injected per kg. of rabbit; at the last test, after 30 days, 0.12 cc. was injected per kg. The figures in the column under "Result" indicate the number of days which elapsed before the animal died. Strain No. 1036.

	PH.	Result	PH.	Result	PH.	Result
Original bouillon	6.4		7.5		8.4	
After 6 days	6.8	lives	7.2	lives	7.8	lives
After 8 days	6.8	»	7.7	5 +	8.1	3 +
After 15 days.	7.2	»	7.9	3 +	8.4	4 +
After 20 days.	7.7	2 +	8.1	2 +	8.4	2 +
After 30 days 0.18 per kg. . .	8.4	3 +	8.5	2 +	8.5	2 +
0.12 per kg.		lives		3 +		2 +

(1) Doerr has already shown that, without the admission of air, the toxins are not formed.

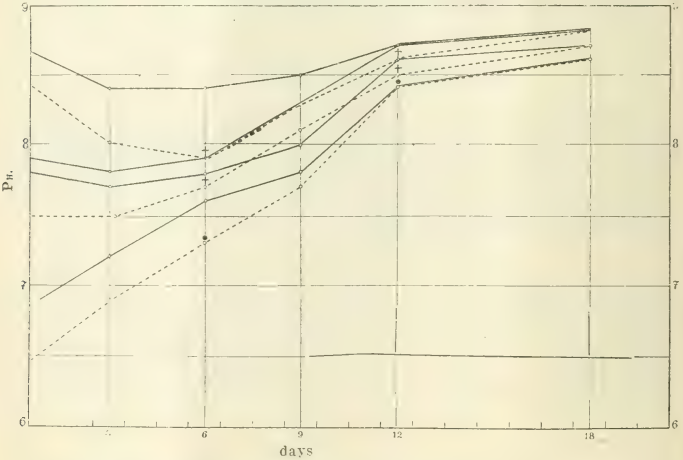
Curve No. I.

Relation between toxicity and alkalinity of the nutrient medium. Strain No. 25. Depth of the medium 1 cc.



Curve No. II.

Relation between toxicity and alkalinity of the nutrient medium. Strain No. 17. Depth of the medium 1 cm.



— Toxicity and Ph content tests. † Dead.
— Ph content test only. • Survived

This experiment shows that toxins are formed in acid as well as in neutral or alkaline nutrient media, with the sole difference that their presence can be demonstrated after a longer or shorter period of time. As stated above, the acid bouillon became alkaline, but the reaction only rose above the neutral point at the end of 20 days. While earlier tests did not show any sign of toxicity, examination of the sample at the end of 20 days showed at Ph. 7.7 virulent toxins and the animal died after two days. With an original bouillon of Ph. content 7.5, the nutrient medium first becomes increasingly acid. After 6 days, Ph. is of 7.2, and the sample contains no toxin. After 8 days, the Ph. reaches 7.7 and toxicity sets in. With an original bouillon of 8.4, Ph. content is 7.8 after 6 days, therefore slightly alkaline, but no toxins can be found in the quantities employed. An accurate examination of the Ph. curve shows, however, that the nutrient medium is in process of acidification. After 8 days, alkalescence is more marked and the filtrates are toxic. We may conclude from these facts that *the toxicity of the filtrates depends not only on the Ph. content of the nutrient medium at the time but also on the alkalisation of the medium as a result of growth.*

It results, therefore, that the essential condition for the formation of toxins is that Ph. content should exceed Ph. 7.5. This degree of alkalescence develops in acid as well as in neutral nutrient media. The question as to when this change in the reaction takes place, depends, as stated, on secondary factors. It is most easily regulated by varying the depth of the nutritive medium. At a slighter depth of the bouillon, alkalisation develops more rapidly than when large quantities of bouillon are used. It is not sufficient, therefore merely to indicate after how many days the toxicity test was carried out, without giving due weight to the factors we have mentioned. In more recent experiments we have, therefore, measured the quantity of bouillon in such a way that the depth of the nutrient medium amounted to only one 1 cm. and toxicity was tested after 6 and 13 days. In carrying out the experiment according to this method, the nutrient media were shown to be in process of increasing alkalisation at the end of 6 days. The maximum in Ph. was reached after 13 days.

Table III (*abridged*).

Original bouillon: Ph content 6.4, 7.5 and 8.4. Several doses of every sample were injected into rabbits, *i.e.* 0.5, 0.3, 0.15 and 0.1 cc. Cases in which the animal, after injection, showed symptoms of disease, but survived, are marked in the tables with the sign >. The figures in the column headed "Result" represent toxic doses per kg. of rabbit.

	Ph.	Result D. L.	Ph.	Result D. L.	Ph.	Result D. L.
Original bouillon	6.4		7.5		8.4	
After 6 days.	7.3	0.3	7.9	0.15	7.9	>0.15
After 13 days	8.3	>0.15	8.6	0.15	8.7	>0.15

This experiment also showed that toxins can form in acid as well as in neutral bouillons. There are unmistakable quantitative differences, however; more virulent toxins having formed at Ph. 7.5 than at Ph. 6.4. When alkaline bouillon was used, the injection of 0.15 cc. produced symptoms of disease, but the animal survived. This experiment showed that alkalisation, and therefore toxicity also, developed more rapidly than in experiment II. The most suitable degree of alkalescence was Ph. 7.5.

Table IV (*abridged*).

Technique and designation as in Table III. Strain No. 25.

	Ph.	Result D. L.	Ph.	Result D. L.	Ph.	Result D. L.
Original Bouillon	6.4		7.5		8.4	
After 6 days	7.5	0.15	7.8	0.15	8.4	0.3
After 13 days.	8.3	0.15	8.5	0.1	8.5	>0.1

This experiment showed similar results throughout. After 6 days the reaction rose above the neutral point and the toxins had already formed. It is worthy of note that the toxins were more virulent in the case of acid original bouillon than in the case of strongly alkaline bouillon. Ph. of the most suitable original bouillon was again shown to be 7.5.

Table V (*abridged*).

Technique and designation as in Table III. Strain No. 17.

	Ph.	Result D. L.	Ph.	Result D. L.	Ph.	Result D. L.
Original bouillon	6.4		7.5		8.4	
After 6 days.	7.3	»	7.8	0.1	7.9	0.15
After 13 days	8.5	lived	8.5	0.1	8.6	0.15

Toxicity did not develop when acid bouillon was used with this strain. The most suitable condition for toxicity was Ph. 7.5 in this experiment also.

We have carried out further experiments in which we added diluted white of egg in accordance with Olitzky's and Kligler's method. We found no difference in the degree of toxicity of the bouillon, whether white of egg was used or not.

Summing up, we may say that the formation of dysentery toxins is possible in acid, neutral or alkaline original bouillon and, in particular, during the period of increasing alkalisation, when the reaction exceeds Ph. 7.3 to 7.5. The time required for the formation of toxins in the bouillon accordingly depends upon the degree of alkalescence, an this can be regulated by varying the depth of the nutrient medium. If a layer of bouillon of a depth of 1 cm. is used, an incubation period of two weeks would appear to be the most favourable, although the presence of toxins can be demonstrated even before the expiration of that period.

II. Regarding the Virulence of Bouillon Toxins, or Extracts of Bacteria and of Living Bacteria

There is more or less general agreement regarding the symptomatology of dysentery intoxication in rabbits. The clinical symptoms are, first of all, paralysis and diarrhœa, pathological anatomical inflammatory œdema in the appendix and the colon, submucous extravasation of blood, hæmorrhagic infiltration and, finally, necrosis and ulceration. On two questions, however, agreement has not yet been reached, *i.e.*, whether the various symptoms are to be attributed to one and the same constituent of the toxin and whether the constituent which causes dysentery in human beings is the same as the one which causes paresis in rabbits, — a question which is extremely important from the point of view of human pathology and the technique of standardisation. Kraus and Doerr, Todt, Rosenthal have obtained typical toxins from dysentery cultures; but Pfeiffer and Ungermann are of opinion that the presence of two different toxins must be assumed, one of which particularly affects rabbits and the other guinea-pigs. As regards dysentery in human beings, only the toxin from guinea-pigs would appear to be of importance and the testing of dysentery sera on rabbits would, accordingly, seem to be an experiment made on an object useless for the purpose. This binary division of the dysentery toxin was denied by Bächer and Daub. Kolle, Heller and Mestral, on the other hand, assume the presence of endotoxin as well as of toxin; the toxin in the body of the bacterium is supposed to contain both toxin and endotoxin. Welter is of opinion that toxin from rabbits does not affect human beings and that anti-toxins against toxin from rabbits are of no value in human pathology. According to Dessau, it is chiefly the endotoxin which is of value for human beings. Horimi even goes so far as to assume the existence of various toxins and endotoxins showing an affinity to various organs, and he believes that pathological anatomical differences must be assumed in the action of the various constituents of the toxin.

From the point of view of the technique of standardisation, the experiments made by Olitzky and Kligler were of great value. The authors of the experiment believe that the formation of neurotoxin must be attributed to the action of toxins and the intestinal symptoms to the action of endotoxins. Several of the sera from American authors neutralised the neuro-toxins when used in a more diluted form than was required for enterotoxins. This leads us to the conclusion that the simultaneous titration of the anti-toxins and anti-endotoxins contained in serum is a practical necessity. Olitzky and Kligler observed that young bouillon cultures contain chiefly toxins and produce nervous symptoms; that heat destroys the thermolabile toxins, and that pure endotoxins, which only produce intestinal symptoms, may therefore be obtained by heating older bouillon-cultures.

It appeared to us that the adoption of a particular standard serum would not be advisable until these far-reaching statements had been controlled. If, for example, a particular standard anti-toxin only neutralised neuro-toxins without reacting to endotoxin, i.e., *intestinal toxin or without exercising anti-infectious action, we should have adopted, from the very beginning, an inaccurate standard for our anti-toxic sera.*

For this reason we resumed our study of dysentery toxins and, in particular, we further investigated the statements of Olitzky and Kligler. 10 other strains were inoculated into bouillon at Ph. content 7.5—7.6. The depth of the nutrient medium was 1 cm. We employed Roux bottles, which we placed in the necessary slanting position. Samples were taken after 6, 12 and 18 days and sterilised by the addition of phenol; after 24-48 hours the bacteria were centrifugalised and the toxin was titrated by means of rabbits. In order to exclude the individual sensitiveness of animals, we have always injected several rabbits with the same sample.

Among the chief symptoms must be placed paresis of the anterior or posterior extremities. The animals generally lie on their chest without support from the anterior extremities, or slightly on the side, often with their hind legs stretched out. We designate paresis of the fore-legs in the tables as V.P., paresis of the hindlegs as H.P., and symptoms leading up to general paralysis as a. P. In the case of post-mortem examination in rabbits inoculated with dysentery we often find a full urinary bladder, sometimes distended to its utmost capacity, as a result, no doubt, of paralysis of the muscular walls of the bladder. Sometimes we observe a full bladder even when other symptoms connected with the nervous system are absent. In such cases the sign N+— is used in the abridged tables. We employ the sign N+ for marked paresis of the fore- or hind-legs and N++ for general paralysis.

We have seldom obtained by means of toxins the clinical dysentery symptoms, such as blood or mucous in the fæces or diarrhœa. The fact that other authors and our colleagues in Cracow have more frequently obtained such symptoms may be due to the use of stronger doses of toxin. Vascular injections of the cæcum and colon are the first stage which can be anatomically determined. We designate this condition as +—. This state is not particularly characteristic and it would be impossible to base a diagnosis upon it (1). As the toxication increases further, circumscribed œdema may be observed, the mucous membrane is swollen and the intestinal wall somewhat inspissated. This stage is shown by the sign +. If intoxication is more pronounced, we notice slight or extensive hæmorrhages, occasionally small blood foci, which are only visible if the intestinal wall is held against the light, and sometimes more serious bleedings which uncover the mucous membrane. This stage is noted as ++. When,

(1) In discussing the detailed tables we have not taken these unimportant conditions into account.

the intoxication is at its highest, we see the typical symptoms, *i.e.*, gelatinous œdema, hæmorrhages, necrosis and, finally, slight or more extensive ulcerations. The sign +++ denotes this stage. In our tables we note either the clinical symptoms or the intestinal change which can be observed with the naked eye. Professor Hornowski, Director of the Pathologico-anatomical Institute, Dr. H. Sparrow and Mr. Hirszberg (medical student) undertook the microscopic work.

In all the experiments which we undertook, there were a number of animals which died within 24 hours, sometimes immediately after the injection, and sometimes after a few hours. Cases occur in which rabbits, after receiving a small injection, die within a short time, while most of them can support stronger doses. The letter S in the results of experiments denotes sudden death. These cases cannot be attributed to the action of peptone or meat extract, since much stronger doses of the bouillon alone have no effect; bacterial extracts on the other hand also cause rapid death. As regards 10 strains, which were employed in the following experiments, sudden death was observed in the case of 16% of the rabbits. We do not find pathologico-anatomical changes in these cases—at most vascular injection and sometimes hæmorrhage as well. It was impossible, in view of the varying sensitiveness of the animals, to carry out an accurate investigation of these quick-working toxins. It has been proved by the experiments in the titration of dysentery sera, which we conduct in large numbers in the Institute every year, that these rapid poisons cannot be neutralised even by good sera. We have observed a great number of cases in which well neutralised toxin mixtures caused several animals to die within a few hours. The toxins which cause rapid death have therefore nothing to do with dysentery toxin, which can be neutralised, and should not be taken into account in connection with the titration of dysentery sera.

Even in the case of considerable changes in the cæcum, we found in the small intestine at most the uncharacteristic vascular injections which are so difficult to interpret in connection with the spread of intestinal coccidiosis. We, therefore, do not consider that we are justified in postulating the existence of a special toxin of the small intestine, according to the view held by Horimi.

In the following experiments the bouillon was throughout brought to Ph. 7.5 and the toxicity of samples taken was examined after 6, 12 and 18 days. We gave special attention to the question whether the demonstrable toxins in young bouillon filtrates produce chiefly neural symptoms and those in old filtrates chiefly intestinal symptoms. Our experiments, however, do not confirm the statements of Olitzky and Kligler. After 6, 12 or 18 days we observed the presence of the neural symptoms with almost unfailing regularity, and, as Doerr rightly pointed out, the most pronounced intestinal symptoms occur in those animals which remain alive longest. It may happen that the same sample produces chiefly neural symptoms in the case of one rabbit and chiefly intestinal symptoms in the case of another. The epitome of our

experiments, which we give in an abridged form, and shows the presence of intestinal symptoms to be irregular. As regards many animals, it is clearly the intestinal system which is sensitive, while as regards others it is the neural system.

If in reality the toxins only caused intestinal symptoms, we should discover their presence chiefly in connection with the injection of extracts; and in fact we observed the most marked symptoms of an intestinal character on injecting extracts. We therefore gained the impression that extracts strengthen the action of the toxins, but are not the sole cause of the intestinal symptoms. In injecting typhus and cholera extracts we have, indeed, also noticed intestinal changes such as severe vascular injections, œdema, and sometimes even slight hæmorrhage—changes which were to some extent similar to those caused by the extracts of dysentery bacilli, but which never reached the stage of ulceration or gelatinous œdema. A combination of the specific action of dysentery toxin and of the unspecific irritation of bacterial proteins might therefore explain why we observed more marked changes when employing extracts.

The experiments regarding the separation of the toxins from the endotoxins by raising the temperature to 75°, as suggested by Olitzky and Kligler, likewise produced no definite results. In the case of Strains Nos. 10 and 100, the heated extracts caused intestinal symptoms rather than neural symptoms, and in the case of Strain No. 2 the position was reversed. The results are in any case so irregular that the "pure" endotoxins produced by heat could certainly not be employed in the technique of standardisation.

To sum up, the experiments prove that both young and old bouillon toxins contain in solution the two constituents which are dangerous for rabbits, *i.e.*, neural and intestinal toxins (1). As a rule smaller quantities of bouillon toxins are required to be more effective than of bacterial extracts. For the practical purposes of testing, therefore, toxins in solution can be recommended.

III. Neutralising Properties of Immune Sera produced by the Injection of Filtrates of Young Bouillon Cultures or by the Injection of the Bacteria Themselves

Former experiments showed that the symptomatology of dysentery intoxication in rabbits is the same, whether the samples are taken after 6, 12 or 18 days. Though when extracts were employed the intestinal symptoms were more marked, yet the experiments were by no means so unambiguous as to prove affinity between a specific endotoxin and the intestinal mucous membrane.

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(1) The different strains do not greatly differ from each other in this connection.

EPITOME OF EXPERIMENTS REGARDING THE TOXICITY OF FILTRATES AFTER 6, 12 AND 18 DAYS, OF EXTRACTS AND OF LIVING BACTERIA

(Abridged)

Bouillon toxins

Number of days	Experiment No. 6			Experiment No. 7		
	Ph.	Dose per kilog.	Result	Ph.	Dose per kilog.	Result
6	7.7	0.2	4 N+ D—	7.7	0.2	7 N++ D+—
		0.2	7 N+ D—		0.1	S.
		0.1	4 N++ D+—		0.1	7 N++ D+—
		0.1	S		0.1	4 N+— D+—
		0.1	4 N+— D—		0.1	5 N—— D+—
		0.05	6 N+ D++			
12	8.4	0.15	2 N— D+—	8.4	0.15	3 N+ D+—
		0.1	S		0.1	3 N+ D+—
		0.1	1 N— D—		0.1	2 N— D+—
		0.1	2 N— D—		0.05	2 N+ D+—
		0.05	2 N— D—		0.05	S
		0.05	Survived		0.01	Survived

N = neural symptoms
D = intestinal symptoms

EPITOME OF EXPERIMENTS (*continued*)

	Number of days	Experiment No. 6			Experiment No. 7		
		Pit.	Dose per kilog.	Result	Pit.	Dose per kilog.	Result
Bouillon toxins	18	8.9	0.1	2 N− D+−	8.9	0.1	Survived
			0.05	3 N+ D+−		0.1	3 N+ D+−
			0.05	5 N+ D−		0.05	S
			0.01	6 N+ D−		0.05	4 N++ D+−
						0.01	10 N+− D+−
Extracts	Unheated	Filtrated	0.3	4 N++ D+−	Filtrated	0.2	2 N+− D++
			0.1	2 N++ D+−		0.15	Survived
			0.2	S			
		Centrifugalised	0.1	N++ Survived			
			0.05	9 N++ D+++			
	Heated	Filtrated	1.0	3 N− D+−	Filtrated	0.5	5 N+ D−
			0.5	1 N− D−			
Living bacteria			1/3 Agar culture (Subcutaneously)	2 N+− D+++		1/3 Agar culture (Subcutaneously)	5 N++ D+++
			»	2 N+− D++		»	
				Strain No. 31			Strain No. 2

EPITOME OF EXPERIMENTS (*continued*)

	Number of days	Experiment No. 8			Experiment No. 9		
		Ph.	Dose per kilog.	Result	Ph.	Dose per kilog.	Result
Bouillon toxins	6	7.7	0.2	Survived	7.7	0.2	Survived N+
			0.1	7 N++ D−		0.1	7 N+ D+−
			0.1	4 N− D+−		0.1	Survived
			—				
	12	8.0	0.15	3 N+ D++	8.0	0.15	S. ? D+−
			0.1	3 N++ D+−		0.1	2 N++ D−
			0.1	2 N− D−		0.05	S.
			0.05	6 N++ D++		0.05	3 N++ D++
						0.01	Survived
	18	8.8	0.1	1 N− D−			
			0.05	2 N+ D+−			
			0.05	3 N− D−			
			0.01	Survived			
Extracts	Unheated	Filtrated	0.2	2 N− D++			
	Heated	Filtrated	0.5	2 N− D−			
			0.3	15 N− D+−			
Living bacteria			1/3 Agar culture (Subcutaneously)	4 N+ D++		1/3 Agar culture (Subcutaneously)	3 N− D++
						»	5 N+ D++
		Strain No. 61			Strain No. 2675		

EPITOME OF EXPERIMENTS (*continued*)

	Number of days	Experiment No. 10			Experiment No. 11		
		Ph.	Dose per kilog.	Result	Ph.	Dose per kilog.	Result
Bouillon toxins	6	7.3	0.2	Survived	8.2	0.15	3 N + − D − −
			0.1	»		0.1	2 N + D + −
						0.1	6 N − D +
						0.05	2 N − D + +
	12	8.4	0.15	2 N − D − −	8.6	0.1	2 N − D −
			0.1	2 N − D −		0.05	3 N − D −
			0.1	3 N − D +		0.01	Survived
			0.1	S.			
			0.05	9 N − D + −			
	18	8.8	0.1	2 N − D + −	8.8	0.05	2 N + D + +
			0.05	10 N − D + −		0.01	Survived
Extracts	Unheated	Filtrated	0.2	1 N − D −			
			0.15	5 N − D −			
	Heated	Filtrated	0.5	Survived			
			0.3	7 N − D −			
Living bacteria			1/3 Agar culture (Subcutaneously)	3 N + + D −		1/3 Agar culture (Subcutaneously)	6 N − D − − −
			»	13 N − D + −			
			Strain No. 44			Strain No. 641	

EPITOME OF EXPERIMENTS (*continued*)

	Number of days	Experiment No. 12			Experiment No. 13		
		Ph.	Dose per kilog.	Result	Ph.	Dose per kilog.	Result
Bouillon toxins	6	8.3	0.15	2 N− D−	7.8	0.15	2 N+ D−
			0.1	3 N− D−		0.1	2 N++ D−
			0.075	2 N++ D++		0.05	1 N− D−
	12	8.7	0.1	2 N− D−	8.5	0.1	2 N− D++
			0.05	16 N+− D+−		0.05	2 N− D++
						0.01	1 N− D+−
	18	8.6	0.1	Survived	8.7	0.05	2 N++ D+
			0.05	Survived N−−D?		0.01	Survived
Extracts	Unheated				Centrifugalised	0.3	1 N++ D+
						0.2	S.
						0.1	11 N− D+
						0.1	Survived
						0.05	2 N− D−
						0.05	Survived
	Heated				Centrifugalised	0.5	2 N− D−−
Living bacteria			1/3 Agar culture (Subcutaneously)	7 N− D++		1/3 Agar culture (Subcutaneously)	3 N− D−−
							5 N+− D+−
		Strain No. 1260			Strain No. 100		

EPITOME OF EXPERIMENTS (*continued*)

	Number of days	Experiment No. 14			Experiment No. 15		
		Pu.	Dose per kilog.	Result	Pu.	Dose per kilog.	Result
Bouillon toxins	6	7.8	0.15	4 N++ D−−	8.0	0.15	S.
			0.1	2 N− D−		0.1	3 N++ D++
			0.05	Survived		0.05	3 N+ D+−
	12	8.2	0.1	4 N++ D+−	8.4	0.1	S.
			0.05	6 N+ D−		0.05	7 N− D−
	18	8.4	0.1	S.	8.6	0.1	Survived
			0.1	Survived		0.05	11 N− D−
			0.05	2 N− D+−			
Extracts	Unheated	Centrifugalised	0.2	S?	Filtrated	0.3	8 N− D++
			0.1	2 N++ D−			
			0.05	14 N− D−			
					Filtrated and heated	1.0	3 N− D++
Living bacteria			1/3 Agar culture (Subcutaneously)	5 N++ D++		1/3 Agar culture (Subcutaneously)	5 N++ D+
				Strain No. 8			Strain No. 10

Epitome of Tables 6-15.

	After days	Nerve symptoms only	Intestinal symptoms only	Nerve and intestinal symptoms	No nerve or intestinal symptoms	Death in a short time	Total
Bouillon toxin	6	17	1	3	6	3	30
	12	9	3	4	12	6	34
	18	7		2	8	2	19
Unheated extract.		4	4	1	4	3	16
Heated extract . .		1	2		5		8
Living bacilli . . .		1	6	6	1		14

The above summary takes no count of the less distinct symptoms, such as vascular injection or "dilation of the bladder". We have not observed that the older toxins cause stronger or more frequent intestinal symptoms than the young toxins. It is true that, in these experiments, the alkalisation and therefore the formation of the toxin—proceeded rapidly, since the layer of bouillon was only 1 cm. deep. In the experiments which we described in Chapter I, thicker layers of bouillon were employed in some cases, and then the formation of toxin took place slowly. As is seen from our very detailed reports, the results were very similar in all cases. We only desire to point out that the action of the extracts produces stronger intestinal symptoms, and that the most frequent and most pronounced changes, both in the nervous and intestinal systems, are caused by infection with living bacteria. When heated extracts are used on rabbits they usually die without any nervous or intestinal symptoms.

As we had so far not succeeded either by using old cultures, or by heating them, in establishing with certainty the distinction alleged to exist between the action of endotoxins and toxins, we endeavoured, by means of serological experiments, to separate these two constituents. The younger bouillon-cultures do, it is true, produce both nervous and intestinal symptoms. But if we are correct in making the distinction referred to above, we should expect to find more toxins in a young culture,—and we ought to be able, accordingly, to prepare antitoxic sera mainly by means of injections of filtrates from young cultures. On the other hand, we know that anti-endotoxins are best prepared by means of intravenous injections with the bacteria themselves. The latter method is used by Professor Bujwid, at Cracow, in Poland. At the Epidemiological Institute at Warsaw, horses are treated, according to the well-known method, with subcutaneous injections of the toxin and intravenous injections with weakened or dead bacteria. We determined to prepare, in addition, a relatively "pure" antitoxic serum, and then to compare these three

sera with each other. If it is true, as Olitzky and Kligler assume, that antitoxin only neutralises the neurotoxin, then experiments with the last-named serum should produce the same results as are related by the American authors, namely, that the neutralised mixture of extracts and old bouillon-filtrates only produces intestinal symptoms. On the other hand, such a serum would be a suitable means for testing whether an antitoxic serum possesses the same curative power as it shows in neutralisation experiments. If anti-bacterial or anti-endotoxic action is an essential condition for curative power, we should expect that sera prepared with living or dead bacteria should produce a more marked *curative*—in contrast to an *antitoxic*—action than the purely antitoxic sera.

The antitoxic serum was prepared in the following manner : weak alkaline bouillon was poured into shallow flasks; the depth of the layer of nutrient medium was 2 cm. The cultures were kept for 6 days at 37°; 1/2% phenol was then added and they were then filtered through a Berkefeld filter. The toxin, when injected into rabbits, caused death within 2 days, accompanied by nervous symptoms. The horse "Lak" received once in September 1921, 2 cc. of toxin a fortnight old, which had not been filtrated. The immunisation with "six days old" toxins was carried out once a week in increasing doses, viz : 3, 5, 8, 15, 25, 35, 50, 70, 100, 125, 150, 175, 200, 220, 240 and 250 cc. After being treated in this way, a quantity of 0.003 of the serum was able to neutralise 5 lethal doses and to agglutinate 1 : 200. On March 2nd, 1922, blood was taken and the serum was ready to be employed as "antitoxic Lak serum".

Preparation of the anti-endotoxic serum Dzwonek : 14-day-old bouillon cultures, killed with 0,5% phenol and injected subcutaneously once a fortnight in doses increasing from 1 to 200 cc. In addition, the horse received, every other week, 10 mm., rising to 150 mm. bacterial-substance, injected intravenously, so that the animal received the toxin one week and the bacterial suspension the next week. The immunisation began on August 24 th, 1921, and blood was taken on April 17th, 1922.

Preparation of sera by Bujwid's method : living bacteria are injected intravenously every 5th day, beginning with 2 mm and rising rapidly to 400 mm. The serum is taken after 8 weeks. The Bujwid sera are frequently tested in the Institute, and are found, as a rule, to contain a sufficient quantity of antitoxins.

In Poland, sera prepared in the country are chiefly employed. The doctors have considerable experience with both sera, which they regard as equal in value, and which they find very satisfactory.

In experiments 16 and 17, the Lak antitoxic serum and the Bujwid anti-endotoxic serum were tested, as regards their neutralising properties, in comparison with 18-day bouillon filtrates and bacterial extracts.

In experiment 16, 10 lethal doses of the toxin (lethal dose = 0.05) and 10 lethal doses of the extract from strain No. 100 (lethal dose = 0.05 per kg) were employed. To prepare the extract, a "Roux" bottle was first of all rinsed with 50 cc. NaCl; a few

drops of chloroform were then added, and the bacterial suspensions, after being kept for 6 weeks, centrifugalised in cold storage.

The toxin and the antitoxin mixtures were kept at first for half-an-hour in an incubator, and were then injected, intravenously, into rabbits.

Table No. 16 (*abridged*).

	Serum dose	Toxin Result	Extract Result
Antitoxic serum Lak	0.001	4 N++ D+++	S.
	0.003	Alive N+	4 N+ D+
	0.005	Alive	2 N+ D+++
	0.01	—	Alive
Anti-endotoxic serum Bujwid 980″.	0.001	S	2 N+— D+—
	0.003	S	1 N— D+—
	0.005	Alive N+	1 N— D+—
	0.01		2 N— D—
	0.02		Alive

Note : The figures under the heading "Result" indicate :
The day on which death took place.
Designation : See text.
Strain No. 100.

This experiment shows that a quantity of 0.01 cc. of "antitoxic serum" neutralises the extracts, and that a quantity of 0.005 cc. neutralises the toxins. It requires a quantity of 0.02 cc. of the anti-endotoxic serum to neutralise the extracts, a quantity of 0.01 cc. is not sufficient. The anti-toxic serum, if titrated with extracts or with toxins, is found to be twice as strong as the Bujwid serum. The extracts and toxins cause the usual symptoms of intoxication. Mixtures which have been incompletely neutralised by "immune sera" give rise in many cases to nervous, and, in many other cases, to intestinal symptoms. The toxins, when mixed with 0.001 cc. of the "immune serum" cause both nervous and intestinal symptoms and finally death. When doses of 0.003 cc. are used, we notice temporary paresis without diarrhœa. *We do not observe that the antitoxic serum only acts on the nerve toxin and leaves the constituents affecting the intestines unchanged.* The transition from insufficient doses of serum to doses which neutralise completely does not show any zone in which only intestinal symptoms are apparent.

Table No. 17 (*abridged*).

Test of the "Lak" antitoxic serum, and of the Bujwid anti-endotoxic serum, No. 898

Technique and designation as in Report 16.
Extract from strain No. 8 D. L. 0. 1 cc.
Toxin from 18-day bouillon filtrates of strain No. 31 D. L. 0. 05.

	Serum dose	Toxin Result	Extract Result
Antitoxic serum Lak .	0.001		3 N++ D++
	0.003	S? N— D++	4 N++ D+
	0.005	2 N++ D+++	3 N— D++
	0.01	5 N++ D++	Alive
	0.015	Alive	
Anti-endotoxic serum Bujwid No. 898. .	0.001		S? N— D+
	0.003	3 N++ D+	2 N+— D+
	0.005	3 N++ D++	3 N++ D+++
	0.01	3 N+— D++	Alive
	0.015	Alive	

The anti-endotoxic serum 898 is seen to be as strong in this experiment as the "Lak" serum. 0.01 cc. of the "Lak" serum and the Bujwid serum neutralise 1 ccm. of the extract; and 0.15 of both sera neutralise 1 cc. of the toxin employed. Again in this case, both sera show the same affinities to toxin and extract. *There is no evidence that the Bujwid serum possesses any greater neutralising power against extracts* (*intestinal toxins*) *or that the Lak serum possesses any such power against toxins* (*nerve toxins*). A dose of serum just too small to be effective (0.01 cc.), if mixed with toxin, causes both intestinal and nervous symptoms. In the case of extracts, we noticed, indeed, that the antitoxic serum neutralised the nerve constituent, but we also noticed similar phenomena with the anti-endotoxic Bujwid serum when toxin was employed. We therefore conclude that the anti-endotoxic serum has the same power as the antitoxic serum for neutralising intestinal toxins and neuro-toxins.

If we compare the two sets of experiments, we find that :

(*a*) Immunisation with dysentery bacteria causes antitoxins in the blood capable of neutralising neural toxins.

(*b*) Immunisation with filtrates from young bouillon cultures produces substances which are capable of neutralising the action of toxins on the intestines.

Mixtures which are not completely neutralised sometimes produce intestinal and sometimes nervous action; but the results are by no means sufficiently constant to justify the conclusion that the neutralisation of neuro-toxins by anti-toxins leaves the real dysenteric intestinal toxin unaffected.

IV. Neutralising Properties of the Sera when large Doses of Toxin and Extract are employed

In the previous chapter we described the experiments by which we measured the neutralising properties of the sera, according to Rosenthal's method, using 10 times the lethal dose. This

is the ordinary technical method which the Institute has employed for many years past. In the following pages we shall describe experiments in which 20-100 times the lethal dose was employed.

Table No. 18 (*abridged*).

"The antitoxic serum Lak" and "the anti-endotoxic serum Bujwid No. 980" were mixed with 50 lethal doses of the extract of strain No. 100. The extract is prepared as follows : a Roux bottle is rinsed with 50 cc. of water, then shaken for three hours and then placed for three weeks in a refrigerator until the bacteria have been deposited. The lethal dose is 0.15 cc. per kilogramme. 0.005 cc. of the Lak serum completely neutralises 5 lethal doses. 0.005 cc. of the Bujwid serum does not completely neutralise 5 lethal doses. The animal dies after 6 days without showing nerve symptoms but showing slight intestinal symptoms.

	Serum dose	Extract result
Antitoxic serum Lak	0.001	S
	0.005	4 N— D+
	0.05	7 N— D+
	0.1	6 N— D+++
	0.25	Survives
	0.5	Survives
Anti-endotoxic serum Bujwid No. 980	0.001	1 N— D+
	0.005	1 N— D++
	0.05	9 N— D+—
	0.1	6 N— D+
	0.25	2 N— D++
	0.5	7 N— D++

The reports shows that if larger doses of antigen are used, larger doses of serum are required than might be expected according to the law or multiple proportion. Thus 0.005 cc. of the serum neutralises 5 lethal doses, but if 50 lethal doses are employed, we need not 0.05 but 0.25 cc. Experiments with the Bujwid serum did not afford such clear results, as 0.005 cc. of the serum did not completely neutralise the toxin. If doses of extract 10 times stronger are used, even 100 times the dose of serum is ineffective.

Table No. 19 (*abridged*).

The serum Lak and the anti-endotoxic serum Dzwonek were investigated. Technique and extract as in Report 18. The Dzwonek serum, when mixed with the extract, produces strong precipitation.

0.003 cc. of the Dzwonek serum neutralises 5 lethal doses.

	Serum dose	Extract Result
Antitoxic serum Lak	0.01	4 N— D+
	0.05	S
	0.1	10 N— D—+—?
	0.25	1 N— D—
Anti-endotoxic serum Dzwonek	0.01	1 N—D+
	0.05	9 N— D+
	0.1	Survives
	0.25	2 N— D++

This experiment shows that, even when the doses of serum are increased 50 times, the dose of extract sometimes proves fatal. We note the phenomenon, often described, that larger doses of serum prove fatal to the animals, whereas small doses, such as 0.1, still remain effective. We also observe the prevalence of intestinal symptoms.

Table No. 20 (*abridged*).

Technique and designation as before. Extract from strain No. 26.

100 toxic doses were employed.

	Serum dose	Extract Result
Antitoxic serum Lak	0.005	1 N— D—
	0.05	2 N— D—++
	0.1	Survives
	0.25	2 N— D+++
	0.5	Survives

Table No. 21 (*abridged*).

The Dzwonek serum (produced by injection of toxins and qacteria), mixed with 10, 25 and 50 doses of filtrate from 18-day-old bouillon cultures of strain 31, was intravenously injected into rabbits after half an hour.

Toxin dose	Serum dose	Result
10 times	0.005	7 N— D—
	0.01	Survives
25 times	0.0125	8. N— D+—
	0.025	3 N— D—
50 times	0.025	10 N— D—
	0.05	8. N. — D—

Both reports confirm the fact that, when larger doses of antigen are used, the neutralising properties of the sera may become unavailing, and—which is particularly unfortunate from the point of view of the technique of standardisation—the results of the experiments are irregular and arbitrary. Experiment No. 21 is

particularly illustrative. When 10 times the toxic dose is used, 0.01 cc. of serum is sufficient, whereas if 0.005 cc. of serum is used, the animal dies after 7 days. If 25 times the toxic dose is employed and the dose of serum is correspondingly increased, this is followed by immediate death in a case of one animal or by death after three days in another case; in the case of another animal with 50 times the dose, immediate death in one case or death after 10 days in another case. 0.01 cc. of the toxin killed the animal after 5 days, producing nerve and intestinal symptoms. If 10 times the dose is used with a small dose of serum, nerve as well as intestinal symptoms may be observed. If 25 or 50 doses are used, both intestinal and nerve symptoms do not occur or are very slightly marked. We thus gain the impression that both toxins and extracts contain toxin constituents which cannot be neutralised and which kill the animal, often with symptoms which are not typical. It is noteworthy, however, that in experiments with high multiples of the doses of extract, the nerve symptoms are very undefined, but the intestinal symptoms are well marked. These observations may be explained by the assumption of Olitzky and Kligler that our dysentery sera neutralise the nerve-constituents but not the intestinal constituents. We question, however, whether such a sweeping assumption may be drawn from these experiments; our reasons are as follows :

(*a*) Clinical experience has proved the great utility of our sera; it may be asserted, as a result of observation in the case of human beings, that the intestinal constituents of toxins are neutralised on infection.

(*b*) When smaller doses of extract or toxin are used, there is no regularly recurring zone in which intestinal symptoms alone occur, if insufficient doses of serum are applied.

(*c*) Extracts mixed with anti-endotoxic serum produce the same symptoms as those mixed with antitoxic serum. If toxins and endotoxins were different constituents of the toxin with a special affinity to the nervous or intestinal system, the zones of intestinal symptoms alone would inevitably be stronger when antitoxic serum was used. This, however, is not the case.

(*d*) If larger doses of extract are used, the animal often dies without showing definite symptoms in the nervous and intestinal system; the real cause of death therefore remains obscure.

(*e*) As already mentioned, cholera and typhus extracts also cause more or less marked changes in the intestine, such as vascular injections, hæmorrhage, etc.,—a fact which points to the stimulating action of the bacterial proteins which are injected in such large quantities when large doses of extract are employed.

V. Bactericial and Anti-infectious Properties of Dysentery Sera

The Lak antitoxic serum contains only few agglutinins (1 : 200); sera produced by bacteria had a titre of 1 : 1600 and more, while

the antitoxic properties were of about equal strength. We therefore considered it particularly desirable to compare these sera in anti-infectious experiments in order to ascertain : (*a*) whether the curative power of the serum depends upon its antitoxins, and (*b*) whether it is necessary to determine the anti-infectious titre together with the measurement of the antitoxin.

In these experiments we left sera under examination together with living bacteria in the incubator for half-an-hour and then injected them; further, we first injected sera intravenously in decreasing quantities, and then, 3 hours later, injected intravenously living bacteria fro fresh Agar cultures.

Table No. 22 (*abridged*).

The Lak serum (agglutination 1 : 200) and Bujwid serum 898 (agglutination 1 : 1600) diluted to 1 : 100 and mixed in quantities of 0.3, 0.6 cc. and 1 cc. with 0.5 cc. of a Shiga-Kruse *suspension* strain No. 100 of an Agar culture in 10 cc. of NaCl.

	Serum dose	Result
Antitoxic serum Lak.	0.003	2 N—D++
	0.006	2 N—D+
	0.01	Survives
Anti-endotoxic serum Bujwid . . .	0.003	9 N—D— — —
	0.006	10 N—D+
	0.01	Survives
Control.	0.25 Bac.	3 N— D— — —

The report shows that the two sera possess equal anti-infectious properties; 0.01 cc. is sufficient to save animals. In these experiments the serum obtained by immunisation with living bacteria proved to be scarcely more effective than the purely "antitoxic". We also see that agglutination alone does not provide any guide as to the efficacy of the serum. The antitoxic serum only contains few agglutinins, but proves effective against both toxins and living bacteria.

Moreover, we note that we have found sera with but slight antitoxic power, even though their content of agglutinin was as high as 1 : 6400, and which did not contain the quantity of antitoxin which we required.

Table No. 23 (*abridged*).

The Lak serum (agglutination 1.200) and Dzwonek serum (agglutination 1 : 3.200). Technique as in report No. 22.

	Serum dose	Result
Antitoxic serum Lak	0.003	2 N++ D++
	0.006	Survives
	0.01	S
Antitoxic and anti-endotoxic serum Dzwonek	0.003	4 N+ +D+
	0.006	Survives
	0.01	10 N−D−
Control.	0.25 Bac.	4 N++ D+++

The results of the experiment are not so clear, since the animal which was inoculated with 0.001 cc. of the Lak serum died immediately, and the rabbit inoculated with a corresponding dose of the Dzwonek serum died after 10 days without typical symptoms and without bacterial infection (post mortem: pneumonia). We may therefore assume it as probable that in both experiments 0.006 cc. had a protective power. In this experiment also we see that both sera are of absolutely equal value. Animals treated with insufficient doses of serum die simultaneously, showing nerve and intestinal symptoms. We may thus see how difficult it is to use a series of experiments as the basis on which to found extensive deductions. In report No. 22, animals inoculated with bacterial serum mixtures died without any nervous symptoms, but did show intestinal symptoms; these cases would therefore appear to confirm the experiments of Olitzky and Kligler. A second experiment identical with these showed different results in this respect.

Further experiments were carried out to test the anti-infectious properties of sera.

Rabbits were given a preliminary inoculation of 2, 1, and 1/2 cc. of serum in the requisite dilution, giving the quantities of serum shown in the tables, and 3 hours later, a suspension of living bacteria was intravenously injected.

Table No. 24 (*abridged*).

Bacteria : 24-hour-old Agar culture of the strain 1062 in 12 cc., 2 cc. being used.

	Serum dose	Result
Antitoxic serum Lak . . .	0.0025	1 N− D+−
	0.005	2 N− D+
	0.01	4 N++ D+
Antitoxic and anti-endotoxic serum Dzwonek.	0.0025	1 N− D+
	0.005	1 N− D+−
	0.01	6 N− D+

In this experiment the sera were not able to prevent death, but succeeded in delaying it. In the case of both sera 0.01 cc. is necessary for this purpose. The Lak serum, although it contains much fewer agglutinins than the Dzwonek serum, is very little inferior to the Dzwonek serum as regards its anti-infectious properties.

Table No. 25 (*abridged*).

Technique as in report No. 24. The strain 1062, freshly isolated, was employed. In a preliminary experiment, an Agar culture suspended in 10 cc., when injected intravenously to the extent of 0.25, killed a rabbit weighing 1,020 grammes on the third day. In the experiment itself an equal quantity of bacteria did not prove fatal. Animals subjected to a preliminary treatment with serum received 0.5 cc., that is to say, either the exact lethal dose or a little more.

	Serum dose	Result
Antitoxic serum Lak	0.0025	5 N— D++
	0.005	6 N+— D++
	0.01	4 N— D++
Anti-endotoxic serum Bujwid 898	0.0025	8 N+— D++
	0.005	6 N— D+++
	0.01	Survives
Antitoxic and anti-endotoxic serum Dzwonek	0.0025	8 N++— D+++
	0.005	Survives
	0.01	6 N++ D+

This experiment does not provide absolutely definite results, as in the case of the Dzwonek serum, 0.005 cc. proves effective, whereas 0.01 does not. The weight of the animals was equal (1,340 and 1,350 grammes). As the Lak serum, in the doses employed afforded no protection at all, it might be inferred that the "purely antitoxic" serum proves to be of less value in anti-infectious experiments. This, however, is contradicted by the result of the experiment shown in report No. 26. Further, the symptomatology of the disease is interesting. When, for example, 0.0025 of the Dzwonek serum is employed, only slight nervous and pronounced intestinal symptoms appear, whereas with 0.01 the animal dies showing symptoms of general paralysis and comparatively slight intestinal symptoms. It will thus be seen that the occurrence of both symptoms is very arbitrary.

Table No. 26 (*abridged*).

The freshly isolated strain 1062 was used for infection. Agar culture suspended in 10 cc., 0.75 of which was injected. 0.5 cc. of the suspension kills the animal after 6 days, showing nervous and intestinal symptoms.

	Serum dose	Result
Antitoxic serum Lak . . .	0.0025	2 N++ D+
	0.005	4 N— D++
	0.01	Survives

	Serum dose	Result
Anti-endotoxic serum Bujwid 898	0.0025	3 N— D+
	0.005	2 N— D+
	0.01	S.N— D+—
Antitoxic and anti-endotoxic serum Dzwonek	0.0025	Survives
	0.005	»
	0.01	3 N— D+++

Once again the experiment affords very irregular results. While on the one hand, in report No. 25, 0.01 cc. of the Lak serum did not prove an effective protection, it did save the animal in this experiment. The Dzwonek serum, on the other hand, proved effective in smaller quantities, but was ineffective when 0.01 cc. was used. These contradictory results were obtained with the same strain as in experiment 25, although we took care to limit ourselves strictly to a lethal dose. We also give the results of an experiment in which, though the same serum was used, we were unable to demonstrate any anti-infectious properties at all.

Table No. 27 (*abridged*).

Technique as before. For the subsequent infection, larger quantities of bacteria were used than before, *i.e.* 2 cc. of a suspension (24-hour-old Agar culture suspended in 10 cc. NaCl.) 1 cc. control kills in 24 hours, strain No. 1062.

	Serum dose	Result
Antitoxic serum Lak	0.0025	1 N—D+—
	0.005	2 N—D+++
	0.01	1 N—D++
Anti-endotoxic serum Bujwid 898	0.0025	2 N—D++
	0.005	2 N—D+
	0.01	1 N—D+
Antitoxic and anti-endotoxic serum Dzwonek	0.0025	2 N—D+—
	0.005	2 N—D+
	0.01	2 N—D++

This experiment shows that, when larger quantities of bacteria are used, sera in doses which in mixture tests provide clear results, do not show any anti-infectious properties.

The experiments as a whole prove that the anti-infectious power of purely antitoxic sera corresponds to the content of antitoxin, and is not essentially inferior to that of anti-endotoxic sera. In the case of more delicate titrations the determination is difficult on account of great differences in the individual sensitiveness of the animals and also of the variations in the virulence of individual cultures. Series are often irregular, smaller quantities of serum affording greater protection than larger quantities. *We therefore*

consider that, as regards practical control tests, it is both difficult and needless to determine the anti infectious properties of sera.

In the light of these facts, an agglutination test would provide nothing more than an indication, inasmuch as sera which agglutinate satisfactorily are sometimes, though not often, insufficiently antitoxic, whilst those which agglutinate only slightly may prove serviceable.

The sera which we investigated showed, when tested for agglutination, a titre of at least 1 : 200, and, in the case of many sera, as much as 1 : 10,000 or even more.

General Conclusions

As we stated at the outset, we considered it inexpedient to adopt a standard serum without first of all giving adequate consideration to the question of the properties which a standard serum should possess. We were particularly anxious to ascertain whether such a serum should neutralise only the neural action of the toxins or intestinal action as well, and whether it should possess anti-infectious properties in addition. We therefore began by carrying out several important series of experiments with many strains for the purpose of testing the hypothesis put forward by Olitzky and Kligler that it is possible to separate nerve toxins and intestinal toxins and that young toxins contain larger quantities of nerve toxins, while old toxins and extracts are richer in intestinal toxins. Moreover, it ought to be definitely determined whether different sera possess a different neutralising power for nerve toxins and for intestinal toxins.

Our experiments, however, did not confirm the hypothesis of the separation mentioned above and indeed, in the case of rabbits, they revealed great individual differences in the sensitiveness of the systems affected. Sometimes nerve symptoms, and at other times intestinal symptoms, are more pronounced in the case of rabbits into which a dose from the same sample has been injected. The raising of the temperature to 75° also failed to produce any definite results; in several experiments the action on the intestines was more marked, while in others the nerves were affected to a greater extent. We therefore endeavoured to settle the question by serological methods and accordingly prepared sera which, according to their origin, were to be designated antitoxic and anti-endotoxic sera respectively. Experiments on animals with these sera, and with living bacteria, extracts and toxins, revealed no qualitative differences and no special affinities with the particular antigens employed in the injection.

Only in the agglutination test did the anti-endotoxic sera prove more potent. *From these experiments we are able to conclude that the curative value of the serum depends on antitoxins and, moreover, that it is sufficient for the purposes of standardisation to determine the antitoxic properties.* Results which are quantitatively of value

are obtained by means of the mixtures experiment, in connection with which we venture to give the following advice :

(*a*) Excessively large doses of toxin should not be employed. Uniform series of unambiguous results are often obtained by using five or ten toxic doses, while with twenty to fifty lethal doses it becomes impossible to reduce the results to any definite rule, and frequently the animals die without showing any clearly marked symptoms. Sometimes, however, well-defined intestinal symptoms are observed (see pp. 29 to 34).

(*b*) The rabbits should weigh from one to two kilos. Wherever possible the same breed of rabbits should be used throughout an entire series of experiments.

(*c*) When this careful titration is adopted, cases where death occurs within twenty-four hours should be disregarded.

Experiments at the Institute show that uniform series may often be obtained by using mice—a view that was upheld by Kolle, Sachs and Georgi. Nevertheless, we ourselves prefer rabbits for the following reasons. In experimenting on mice we observe nothing but the circumstance of death (we observed the occurrence of paresis only shortly before death), and it should be borne in mind that in these experiments we inject into the system a quantity equal to half the volume of the blood. With rabbits, on the other hand, we usually observe clinical or anatomical symptoms. Our experience is that for purposes of control, it is much more satisfactory and certain to work according to some objective, clinical, anatomical or bacteriological standard, than to deal simply and solely with the fact of death. It should also be added that the mouse is about twenty-fifty times less sensitive than the rabbit, the result being that it is not possible to ascertain the finer gradations. We regard as satisfactory those sera of which 0.003 neutralise *at least* five lethal doses. The clinical results obtained with such sera are very gratifying. It would therefore be possible to adopt as a unit a serum, 0.005 cc. of which would neutralise five lethal doses.

Summing up, we may state that we found the mixture test, using from five to ten toxic doses of bouillon toxins, mostly on rabbits, the most satisfactory. We think it unnecessary to determine the anti-infectious titre. (For the preparation of the toxins, see Chap. I.)

Our work dealt with only a part of the problems involved. The experiments of Kraus on the necessity of determining the curative value of simultaneous but separate injections have not yet been tested. It is, however, above all essential to investigate whether the rules discovered by Ehrlich and Behring governing the changes occurring in toxins and the relations between the binding power and toxicity of old toxins in the case of diphtheria, apply also in the case of dysentery. To the best of our knowledge, only Fukuhara has undertaken this investigation. The Frankfort Institute has a standard serum and a standard toxin. We do not, however,

know whether any accurate analysis of the changes occurring in these toxins has already been made.

We therefore consider it desirable that investigations should be made in the variability of toxins with regard to their fixing capacity and toxicity. Attempts should be made to determine in the case of dysentery toxin the $L+$ *and* L_o- *which first made an exact titration possible for other sera.*

STANDARDISATION OF ANTI-DYSENTERY SERUM

INTERIM REPORT

TRANSMITTED BY THE MEDICAL RESEARCH COUNCIL

Work by Drs. O'Brien, Sudmersen, Eagleton and by Drs. MacConkey and Petrie

Only a brief interim report can be made now upon part of the work which has been done in this subject, namely, that carried out at the Wellcome Physiological Research Laboratories by Drs. O'Brien, Sudmersen, and Eagleton, and that at Elstree by Drs. MacConkey and Petrie.

At the Wellcome Physiological Research Laboratories, one sample of a Frankfort toxin and antitoxin were carefully tested. These tests showed that the toxin had apparently deteriorated and the toxicity was considerably lower than that given in Professor Kolle's paper. Thus, the M.L.D. given by Professor Kolle is 0.015 to 0.02 cc. This, however, failed to kill, and it was not until a dose of 0.05 cc. was reached that mice of 20 grammes were constantly killed. As regards the Frankfort antitoxin, it was found that it protected regularly and satisfactorily. Drs. Sudmersen and Eagleton, in a note in the *Lancet* (1921, 2, p. 1109), describe the method of standardising the dysentery antitoxin, which has been in use at the Wellcome Physiological Research Laboratories for some years, as follows :

1 mgm. of dried dysentery (Shiga) bacilli (which was equal to about 10 intravenous lethal doses for a 1,800-gramme rabbit) was mixed with dilutions of the serum to be tested, and after half-anhour was injected intravenously into a rabbit.

The amount of serum necessary to neutralise the test dose of toxin was called *1 unit.*

On the large scale, serum containing from 20 to 40 units per cc. has been produced; an occasional horse will yield serum of from 5 to 10 times this value. The agglutinating properties did not run parallel to the real protective antibodies. Charts of various horses showed that serum might have an agglutinating titre of 2,000-4,000 while possessing no protective antibody.

Further details given by Dr. O'Brien, in a provisional report, are as follows :

Toxin : This consisted of dried dysentery (Shiga) bacilli—M.L.D. for a 1,500-1,800-gramme rabbit 0.1 mgm. when given intravenously : for a 20-gramme mouse the intravenous M.L.D. was 0.1 mgm.

Standardisation of Toxin : For this purpose 1 mgm. of toxin has been adopted as the test dose. This is equal to approximately

10 rabbit M. L. D. For a mouse the same dose is used for the test dose. This is also equal to 10 mouse M.L.D.

Stability of Toxin : From 1919 a single toxin has been used for all standard tests. At that date, some of the toxin was hermetically sealed in phials. One of these has recently been opened. When this was tested it was found that the M. L. D. and neutralising value of the antitoxin appear to have remained constant, and if there has been any variation it is slight. The dried toxin kept in a bottle which has been opened many times in the last four years has, apparently, lost a considerable amount of its toxicity. The M. L. D. has become two or three times as great, whereas the neutralising value when tested against antitoxin has remained practically constant.

The Relation between Mouse and Rabbit : The general indications are that most of the results of the titration of serum by the rabbit and mouse methods agree very closely. There are, however, some indications that, in certain circumstances, the two methods show some variation. Further work is in progress.

Information received from the Lister Institute at Elstree may be summarised as follows :

A dried bacillary (Shiga) toxin prepared by Dr. MacConkey, some 10 years ago, has remained very stable indeed; it has been stored in a desiccator kept in a cold room; the M. L. D. of this toxin was 0.05 mgm. given intravenously for a rabbit of 1,800 grammes (cf. *British Medical Journal*, Jan. 6. 1917). Every series of tests for standardising antitoxin is accompanied by a M. L. D. test of the toxin, to ensure that it has not deteriorated. 100 M. L. D. are used as a test dose. Rabbits are the animals employed.

As the dried toxin remained so stable, and as there were certain special difficulties in the way of the preparation of a standard antitoxin, the matter was left over, to be taken up at a more convenient season. Some subcutaneous tests were done on mice but were not continued, as rabbits were found to be more convenient. Using *pure "Shiga" toxin* for the immunisation, sera may be obtained each c.c. of which will neutralise from 1,000 to 6,000 M. L. Ds. (rabbit), but a similar titre is much more difficult to obtain when the horses are immunised with the *mixed* Shiga and Flexner strains. Rabbits vary very much in their susceptibility to the test toxin, and at present one cannot test very closely. One must be content with fairly large differences between the various quantities of serum used in a test.

A fresh batch of dried dysentery (Shiga) bacilli (mixed strains) has been prepared by Dr. MacConkey, the M. L. D. of which lies between 0.06 and 0.05 mgm.

Recently a "toxin" has been prepared by precipitating a filtered extract of "Shiga" bacilli with sodium sulphate and collecting, drying and powdering the precipitate. The M. L. D. (for rabbits of about 1,300 grammes) of this dried powder is 0.5 mgm.

Dissolved in water, it yields a clear solution which may be kept at 37° C. for at least one hour without loss of toxicity.

When mixed with serum it—unlike the suspension of dried powdered bacilli previously used—must be allowed an appreciable time for combination to take place, as otherwise a false impression will be given by the results of the tests.

Anti-dysentery serum may be refined and concentrated by the same process as is used for diphtheria and tetanus antitoxins and anti-scorpion serum, and therefore it seems that dysentery antitoxin is, like the other antitoxins, attached to the pseudo-globulin of the serum.

All the anti-dysentery serum sent out by the Lister Institute is refined.

When kept under proper conditions, that is, cold and darkness, anti-dysentery serum retains its potency quite well for at least eighteen months.

DYSENTERY SERUM

REPORT

By K. Shiga

Kitasato Institute Tokio.

The following report is based on the work done under the direction of Dr. Shiga, at the Kitasato Institute for Infectious Diseases, with regard to the manufacture, standardisation and use of dysentery serum. Since Dr. Shiga discovered B. dysenteriæ he has followed his theory closely and made effective application of serum therapy for dysentery epidemics in Japan.

1. — Types of B. Dysenteriæ

The dysentery bacilli are differentiated into two types : namely, acid and non-acid. The differentiation is based upon the fermentation of the mannit. Dr. Shiga, following Dr. Hiss' classification, differentiated dysentery bacilli of five types, selecting those types on the basis of fermentation of carbon-hydroxide and of immunological reaction. The non-acid type is called the original type and the acid type is called the varied type, of which, in turn, four types are differentiated (see Table I).

The relation between the types of dysentery bacilli and their agglutination properties is given in Table II.

The immune serum prepared by using the non-acid type does not react against the acid types, and in a similar manner, the immune serum secured by the acid type does not react against the non-acid types. But even among the related acid types, there are varied degrees of group-agglutination.

There are other classifications. For example, Kraus and Doerr classified the types of B. dysenteriæ into toxic and atoxic forms. Lentz called the latter mild toxic form. Again the acid form is differentiated into four types, but the immunological and biochemical properties of these types are often found in close resemblance, and it is always difficult to distinguish the exact relations of their properties.

What the epidemiological, pathological and clinical relations of these various types of B. dysenteriæ are has not been as yet finally decided. But in general, the epidemic caused by the non-acid type is very virulent and is responsible for many serious cases; on the other hand, an epidemic caused by the acid type has usually a sporadic character and severe cases are not common. It is also noted with interest that during the epidemic both non-acid and acid types were found in persons who developed the disease.

During dysentery epidemics in Japan, marked differences in the micro-organisms became apparent; the types of bacilli varied according to time and place, and particularly was this proved true when Dr. Shiga began to study these epidemics. The dysentery epidemic which broke out in Tokyo previous to 1904 was caused almost entirely by the non-acid type, but it was reported by Dr. Futaki that, during the epidemic which spread through the capital of Japan in 1904, he found, after an investigation of over 100 cases, the non-acid type in 2 cases; the others were all acid types. Again the same year, in the epidemic in Kobe, both types were found almost in equal numbers according to Dr. Amakos' report. In the same year, in Manchuria, Chosen and Port Arthur, the non-acid type was found in the greater number of cases according to Drs. Hata, Momoze, Oshida and Ono. In the epidemic of 1912, which spread in the Prefecture of Kagawa, 83% were of non-acid type.

II. — Direction of Manufacture of Anti-dysentery Serum

Anti-dysentery serum is manufactured by bleeding a highly immunised horse, and the amount of antibody is then tested: when it is found that the serum contains a certain amount of immunity units, it is used for therapeutic purposes.

The growth obtained from one 24-hour agar slope culture is suspended in normal saline of 0.85%. A small dose of this suspension is injected—the amount increasing gradually— into a horse subcutaneously, the bacillus having been killed by an exposure at a temperature of 60 degrees for half-an-hour. After this injection, local swelling occurs, the body temperature increases, and the horse loses appetite; at times diarrhœa is caused. After several days, when this condition passes, an increased amount of 1/3 to 2/3 is injected. When the amount injected is excessive, the seat of the injection becomes suppurative and the animal becomes weak, and it is not possible to obtain effective serum. The serum of a horse immunised by intravenous injection is also effective. For immunity purposes, cultures of high virulence must be used. Cultures isolated directly from a patient are the most valuable for immunity purposes; cultures obtained after passage in an animal cannot prove effective. Dysentery bacilli of high virulence are therefore isolated from a dysentery case and grown as puncture culture on an agar-medium in an ice-box. When required, this culture is reinoculated on agar-slant and the immunisation material is thus manufactured.

When a horse reaches a certain degree of immunisation in 2 to 3 weeks after the last dose inoculated, bleeding is made and the blood placed in a cylinder. After a serum has separated, carbolic acid in the proportion of 0.5% is added and the serum is placed in a condenser for several days.

III. — Standardisation of Anti-dysentery Serum

When the anti-dysentery serum is used for therapeutic purposes in man, it has to meet certain demands. It is standardised in animals and its harmlessness is proved. Dr. Shiga is using mice for the standardisation of anti-dysentery serum. In the case of the highly virulent strain of dysentery bacilli, the M. L. D. (by intraperitoneal inoculation) is 0.1 to 0.08 mg. for a mouse of a body-weight of 12 to 14 g. For the standardisation of serum, five times the M. L. D. (0.4 mg.) is added to a certain amount of serum; this mixture is injected intraperitoneally and the effect is examined after 24 hours in order to determine the standard.

The quantity of serum which measures less than 0.0025 cc. and possesses protective power against five times the M. L. D. is considered effective, and that which fails to possess this strength is considered ineffective. If, therefore, anti-dysentery serum of 1.0 cc., having protective power equal to five times the M. L. D. by 0.1 cc., is a standard unit, the Shiga anti-dysentery serum which is used at present has at least 40 units in 1.0 cc. (40 I. U.).

IV. — Directions for Use of Anti-dysentery Serum

The dose of anti-dysentery serum used for injection depends upon the seriousness of the illness and on the age of the patient. For a mild case, 10.0 cc. is generally used once; for a more serious case, two injections of 10.0 cc., one in the morning and one in the afternoon, are sufficient, and for a grave case, two injections of 10.0 cc. are given daily for two or three days. That is to say, after the two injections on the first day, if the patient becomes worse, the injection is repeated. For children, 5.0 cc. to 8.0 cc., according to age, is used once. The above method may not be found effective; the patient will then become physically weak and the condition of intoxication increases, or there may be another complication. In such cases, it is considered best to adopt symptomatic treatment.

The anti-dysentery serum has bactericidal power as well as antitoxic. The fact that the anti-dysentery serum has a conspicuous clinical effect compared with the other anti-bactericidal serum is due to the fact that it possesses this antitoxic power.

In connection with the clinical application of anti-dysentery serum, the existence of several different types of dysentery bacilli which differ in immunisation reaction has to be taken into account.

For practical reasons it is advisable to use polyvalent serum for practical purposes, as the types of bacilli isolated differ considerably according to time and place. Dr. Shiga has produced polyvalent serum with the bacilli of non-acid and acid types of Nos. 2, 3, 4 and applied it practically.

Todd (10, 11) and Kraus and Doerr (6, 7) manufactured toxin in bouillon culture. By this culture, they immunised a horse and

produced antitoxic serum of high value. It is reported that 20 times .001 cc. of toxin of M. L. D. of this serum protected a young rabbit. But the Shiga anti-dysentery serum which is prepared by injection of a suspension of dysentery bacilli (the suspension is kept in an incubator for 24 hours) has the same antitoxic power and at the same time protects the animal from the infection of dysentery bacilli (*i.e.*, bactericidal power). It is reported that the Todd antitoxic serum has both antitoxic and bactericidal powers (Rosenthal). In other words, the antitoxic serum of Dr. Todd is the same as that of Dr. Shiga (Klein [18] and Shiga). The antitoxic serum of Kraus has a very weak anti-bactericida-power compared with that of Dr. Shiga, and it is also weak in antil toxic power (Megure [24]).

V. — Clinical Effect of Anti-dysentery Serum

When anti-dysentery serum is used in the early period of the disease, it is effective and promptly relieves the symptoms within 18 or 20 hours. After the injection of serum, the dysenteric fæces disappear and diarrhœa ceases; the pain is relieved. When the serum is injected when the disease is somewhat developed, the patient feels better and temperature, diarrhœa and tenesmus are reduced. The local symptoms diminish and appetite and the quantity of urine increase. The period of recovery is within a week.

The reaction of anti-dysentery serum on temperature is remarkable and it becomes normal on the day after injection (Shiga [1] Rosenthal).

It has also a remarkable reaction on the frequency of diarrhœa, and on the day following, the injection of the serum greatly reduces it. The character of the fæces also becomes normal (Shiga [1] Rosenthal [2] Kruse [4]). At the ulceration period, the injection of anti-dysentery serum may not attain the usual effectiveness.

The course of dysentery, which will be on the average from 3 to 4 weeks, is reduced by serum therapy. Even in fatal cases the course of the disease is prolonged. Dr. Shiga confirmed that the serum therapy reduced the course of disease further than that of pharmacological treatment. The result is shown in Table III. This table demonstrates that the course of disease averaged 25 days in the case of serum therapy and 40 days in the case of pharmacological treatment. In fatal cases the average period of disease in the former treatment was 16 days, and in the latter, 11 days.

The death-rate may be reduced by serum therapy from 1/2 or 1/3 as against ordinary treatment (see Table IV).

The prognosis of dysentery depends upon the epidemic, climatic conditions, and seasons of the year. If the frequency of diarrhœa is caused by the rectum infection, its prognosis is better. On the other hand, when the infection is localised in the upper part of the intestine, the frequence of diarrhœa is less, but the toxic

symptoms are severe and the prognosis is a serious one. The result of the investigation made by Dr. Shiga on 371 persons is shown in Tables V and VI.

Table I.

Table showing the Types of Bacillus Dysenteriæ.

Kinds of types		Indol	Culture						
			Dextrose	Mannit	Saccharose	Maltose	Dextrin	Lactose	
Original type	No. 1	None	+	—	—	—	—	—	Shiga bac. Kruse bac. New Haven bacillus are included here.
	No. 2	2% pepton. water. Appeared in 10 days.	+	+	—	—	—	—	
Varied types	No. 3	1% pepton. water. Appeared in 5 days.	+	+	—	—	—	—	Flexner. Strong (Manila bacillus).
	No. 4	do	+	+	—	+	+	—	Harris, Baltimore Wollstein bacilli.
	No. 5	do	+	Changed red. Blue on 4th day.	+	+	+	—	

Table II.

Table showing the Relation between Types of Dysentery Bacilli and their Agglutination Properties.

Serum types of bacillus dysenteriæ	Serum of Rabbits immunised with				
	Non-acid type	Acid type N° I	Acid type N° II	Acid type N° III	Acid type N° IV
Non-acid type . .	100	25	0	5	0
Acid type N° I. .	0	100	100	25	5
» No. II .	0	25	100	12	12
» No. III .	0	25	5	100	50
» No. IV .	0	5	12	25	100

Table III.

Clinical Effect of Serum Therapy compared with Pharmacological Therapy.

Pharmacological treatment			Serum treatment		
Longest period	Shortest period	Average	Longest period	Shortest period	Average
80 days	10 days	40 days	54 days	6 days	25 days
64 days	3 days	11 days	65 days	4 days	16 days

Table IV.

Results of the Serum Treatment compared with those of Pharmacological Treatment.

Year	Place in which treatments were carried out	Treatment applied	Number of patients	Number of fatal cases	Percentage of fatal cases
1897 . . .	Honjo	Pharmacological treatment	336	143	42.4
do . . .	Hiroo	do	4	1	25.0
do . . .	Komagome	do	767	304	39.6
do . . .	Institute for infect. diseases	do	34	8	23.5
1898 . . .	Honjo	do	178	73	42.0
do . . .	Hiroo	do	51	20	37.7
do . . .	Komagome	do	385	141	36.6
1899 . . .	Honjo	do	98	32	32.6
do . . .	Komagome	do	481	105	22.0
1898 . . .	Institute for infect. diseases	Serum treatment	65	6	9.2
1899 . . .	do	do	105	9	8.8
do . . .	Hiroo	do	88	11	12.5
1900 . . .	Tokushima prefecture	do	1,282	250	15.0
do . . .	do	Pharmacological treatment	5,558	1,528	27.5
1913 . . .	Kagawa prefecture	Serum treatment	588	80	13.6
do . . .	do	Pharmacological treatment	38	108	28.4

Table V.

Seat of infection	Pharmacological treatment			Serum treatment		
	Number of patients	Number of fatal cases	Percentage of fatal cases	Number of patients	Number of fatal cases	Percentage of fatal cases
Under-part of Sigmoïdeus . .	62	6	9.7	80	2	2.5
Colon descendens	94	49	52.1	90	8	8.8
Colon transversus	4	4	100	5	0	0
Whole colon	17	15	88.2	7	6	75.0
Colon and ileum	1	1	100	10	4	40.0

Table VI.

Clinical Effect of Serum and Pharmacological Treatment.

Symptoms	Pharmacological treatment			Serum treatment		
	Number of patients treated	Percentage of patients treated by ph. t. to the total number	Number and percentage of fatal cases	Number of patients treated	Percentage of patients treated by s. t. to the total number	Number and percent. of fatal cases
Abnormal psychosis .	30	16.9	25 (83.3½)	22	11.4	11 (50.0%)
Vomiting	24	13.4	20 (83.3%)	16	8.4	9 (57.0%)
Singultus	13	7.3	11 (84.6%)	9	4.7	8 (88.8%)
Sense of opression . .	12	7.0	11 (91.6%)	15	7.9	7 (46.6%)
Subcutaneous hæmorrhage.	8	3.3	7 (87.5%)	1	0.5	1 (100.0%)

The fatality rate of dysentery varies in different years. So far the lowest figure recorded has been 16.5% in 1878, and the highest 30.2% in 1882. The average fatality rate of the disease in Japan has been between 22% and 24%. Table VII shows the number of cases that occurred from 1878 to 1919 and the corresponding number and fatality rate.

Table VII.

Year	No. of cases	No. of deaths	Fatality rate
1878	1,098	1,81	16.5
1879.	8,169	1,477	18.8
1880	5,047	1,305	25.8
1881.	7,001	1,837	26.2
1882.	4,330	1,313	30.2
1883.	21,172	5,066	23.9
1884.	22,524	5,989	26.6
1885.	47,183	10,627	22.5
1886.	24,326	6,839	28.1
1887.	16,149	4,287	26.4
1888.	26,815	6,576	24.6
1889.	22,873	5,960	26.3
1890.	42,633	8,706	20.4
1891.	46,358	11,208	24.2
1892.	70,842	16,844	23.7
1893.	167,305	41,282	24.7
1894.	155,124	38,089	24.5
1895.	52,711	12,959	24.5
1896.	85,876	22,356	26.0
1897.	91,077	23,189	25.4
1898.	90,976	22,392	24.6
1899.	108,713	23,763	22.8
1900.	46,236	10,265	22.1
1901.	69,634	10,889	22.0
1902.	36,985	8,443	22.8
1903.	30,311	7,209	23.7
1904.	22,772	5,166	22.3
1905.	37,988	8,606	22.7

Table VII (*continued*).

Year	No. of cases	No. of deaths	Fatality rate
1906.	22,260	5,135	23.0
1907.	24,926	5,941	23.4
1908.	32,809	7,846	23.8
1909.	28,006	6,836	24.4
1910.	31,961	7,053	22.0
1911.	27,466	6,009	21.8
1912.	25,667	5,721	22.2
1913.	16,779	3,691	22.0
1914.	26,127	5,718	21.8
1915.	21,137	4,368	20.6
1916.	22,454	4,555	20.2
1917.	14,942	3,160	21.1
1918.	13,998	2,823	20.1
1919.	12,915	2,920	22.6

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V — SERODIAGNOSIS OF SYPHILIS

REPORT

BY TH. MADSEN

Abbreviations :

W. R. = Wassermann's test
M. = Meinicke »
S-G = Sachs-Georgi »
Σ = Sigma »

It was impossible to begin the comparative investigations of the four methods for the serodiagnosis of syphilis until the end of April owing to delay in obtaining the necessary ingredients for the Sigma reaction.

The material examined from April to November this year was obtained from four hospitals, with the exception of about 10%, which came from very thoroughly observed private patients.

The W. R., M. and S-G tests were carried out by Dr. *H. Boas*, the Σ test by *J. R. Mörch*, laboratory assistant.

In each case the experiment was made without knowledge of the other results and the clinical diagnosis.

The results appear in the table below, which comprises the figures of the supplementary report presented to the Conference.

Table I.

SERUM

	Number of cases	W. R.	M.	S-G	Σ
S. I.	16	12	7	11	13
S. II	50	49 (1)	44	46	50
S. III.	18	18	16	16	17
S. in treatment.	122	74	57	72	98
S. latent.	338	137	85	133	226
Tabes.	6	5	4	5	6
D. p.	77	77	31	49	77
Syphilis total.	627	372	244	332	487
Controls.	885	0	0	5	3

(1) This negative W. R. occurred in a patient with a very mild recidive.

Spinal Fluid

	Number of cases	W. R.	M.	S-G	Σ
S. II	2	0	0	0	1
S. in treatment.	5	0	0	0	1
S. latent.	7	0	0	0	0
S. cerebrospinal	1	0	0	0	1
Tabes	1	0	0	0	1
D. p.	7	6	4	5	7
Syphilis total.	23	6	4	5	11
Controls (mb. cordis).	2	0	0	0	0

Comparison of the four methods shows the following results :

1. *Control-cases :* The W. R. and M. tests showed quite specific results, no positive reactions occurring in cases without syphilis.

The control material came from 885 patients with different diseases, *viz. :*

Skin diseases, gonorrhœa, ulcus venereum	234
Phthisis and other tubercular diseases	441
Nervous diseases	210

The S-G and Σ tests have given reactions respectively 5 and 3 times in cases in which the most careful investigations gave no indications of syphilis either in the anamnesis or in the clinical symptoms.

They appear in the following table.

Table II.

No.	Sex	Age	Remarks	Clinical diagnosis	W. R.	M.	S-G	Σ units	
1	♀	61	No abort. 2 healthy partus. The ulcers healed without specific treatment	Ulcera cruris	÷	÷	÷	20	Second test 8 days after first.
					÷	÷	÷	÷	
2	♀	17		Gonorrhœa	÷	÷	÷	1.8	
3	♀	21		Psoriasis	÷	÷	÷	5	
4	♂	27		Phthisis	÷	÷	+	5	Second test 10 days after the first.
					÷	÷	+	5	
5	♂	29		Phthisis	÷	÷	÷	2	
6	♂	23		Phthisis	÷	÷	+	÷	
7	♂	11		Meningitis tuberc.	÷	÷	+	÷	
8	♀	28		Gonorrhœa	÷	÷	+	÷	
9	♂			Epilepsia	÷	÷	+	÷	
10	♀	52		Mb. mental.	÷	÷	+	÷	

In the additional report, following case was mentioned : ♂, age 23, clinic. diagn. gonorrh., no sign of syph.

Serological Tests

Date	W. R.	M.	S-G	Σ
26/10	÷	+	+	14
3/11	÷	+	+	30
8/11	auto-inhib.	+	+	14
18/11	÷	+	÷	14
23/11	auto-inhib.	+	÷	14
26/11 (1)	do.	÷	÷	14
» (2)	do.	÷	÷	14
12/12	40.100	+	+	11

On 26/11, two samples of the serum were examined independently. It was at last stated that the patient's mother had syphilis.

Later investigations showed that between the control cases mentioned in Table II, No. 3 and No. 4 had syphilis. Thus the "non-specific" cases are reduced to 5 for the S-G test and to 3 for the Σ test.

In the future investigations, special care should be taken to repeat the serological test in such cases and to follow the patients' clinical fate.

2. *Frequency in syphilis cases.*

Positive reaction was found in :

Serum Σ 77.7 %
W. R. 59.3 %
S-G 53 %
M. 42.1 %

The great superiority of the Σ test as regards positive reactions is shown clearly by the table. This is especially apparent in the groups of syphilis in treatment, and latent syphilis. The Σ test is positive in practically all cases where the other two flocculation tests give positive reaction. The Σ test and the W. R., however, do not run on parallel lines. The Σ is observed earlier in primary syphilis than the W. R.; on the other hand, the Σ test was negative in three cases of syphilis when the W. R. was positive.

The relatively low percentage of S-G test in dementia paralytica is very striking.

3. *Technique.*

(*a*) The performance of the experiments requires the shortest time for the M. and S-G test, a little more for the Σ test, and longest for the W. R. test.

(*b*) *Simplicity.* — The W. R. method is obviously more complicated than the flocculation method, where only two reagents are required. The flocculation methods make it possible to apply one and the same antigen in different institutes and to obtain an international standard.

The greater complexity of the W. R. method can be more easily overcome in a large station than in a small one.

(*c*) *Readings of the tests* are most difficult in the M. and S-G test, whilst the W. R. and the Σ test in its last modification are more decided and therefore much easier.

(*d*) *Time.* — The time required for all the different reactions is shortest in the M. and S-G test, longer in the Σ, and longest in the W. test.

An advantage of the W. R. is that the qualitative reading can be made on the same day as the experiment.

(*e*) *The expense* is considerably less for the three precipitation methods than for the W. R.

(*f*) *The quantity* of serum of patients required

for a		M. test is	0.2	cc.	
—	—	S-G	— 0.4	cc.	
—	quantitative	W. R.	— 1	cc.	
—	—	Σ	— 1.5	cc.	(if the dropping method is used).
—	—	Σ	— 1.0	cc.	(if the pipette method is used).

Technical Remarks.

1. *W. R.* — The technique is exactly as described by H. Boas (*Die Wassermannsche Reaktion*, 3rd edit., Chapter II). It is characteristic of this technique that it works with exact titrated doses of complement, using in the hemolytic system the smallest dose, which, mixed also with antigen, produces total hemolysis. We believe that this exact titration of the complement makes our results compare rather favourably with many of those obtained elsewhere. The method is so adjusted that unspecific results are never obtained.

2. *Meinicke test.* — An advantage is that only 0.2 cc. of serum is required. The reading is not so clear and well defined as that of the W. R. The negative reacting sera are milky and cloudy; the strong positive sera show a quite clear fluid with a heavy precipitate in the bottom of the test-tube, and between these two quite a sliding scale is observed. In some cases the precipitate is so fine that even with a lens it is difficult to be sure of its existence. At this institute, even investigators with great experience have comparatively often disagreed as to whether a reaction should be considered positive or negative; in other words, much more is left to personal judgment than in the case of the W. R. test. A few large flocculi lying in a diffuse milky fluid at the bottom of the test-tube are fairly often observed, but they have no significance,

as they frequently occur in sera from persons who are absolutely free from syphilis.

The reading must be made either with the naked eye or a lens, but not with the agglutinoscope, this instrument showing a fine precipitation in many non-syphilitic sera.

The above results compare unfavourably with those obtained by other investigators. The cause of this may probably be that the extract now employed provided by Dr. Meinicke has become weak. We first thought that the extract received on June 20th, 1922, gave better results than that of January, but continued experiments have shown that it is not so.

3. *Sachs-Georgi.* — The technique is as simple and easy as for the Meinicke test, only 0.4 cc. of serum being required; as in the case of Meinicke, the reading leaves too much liberty to personal judgment. The reaction must be read with an agglutinoscope, the flocculi being so small that they cannot be observed by the naked eye. In the agglutinoscope, all possible transitions are shown from big flakes, very often quite like snowflakes, to very small specks only just distinguishable. This is where the real difficulty occurs; different observers disagree as to the presence of a precipitation; moreover, in quite dependable control sera a few big flocculi can fairly often be seen. The observer must bear this in mind in order to avoid the mistake of considering such sera as positive. For this reason, as in the case with the Meinicke reaction, only thoroughly trained investigators can perform the reaction.

4. *Sigma reaction.* — The amount of serum required for a quantitative Sigma reaction is ca. 1.5 cc. if the dropping method as proposed by *Dreyer and Ward* is used. In using a pipette for this purpose, the quantity of serum can be reduced to ca. 1 cc. (total vol. 0.5 cc.).

The inactivation of serum requires 1 1/2 hours at 56°. This length of time cannot be reduced, as the reaction after 1 hour has not yet reached its full strength. To secure the sterility of serum, we recommend the removal of the coagulum and to perform the inactivation immediately after the reception of the blood. The titration of the reaction is in accordance with *Dreyer and Ward* carried out by the dropping method with a dropping pipette. It requires much practice to carry out this method as quickly as the pipetting; it is no more exact and requires more serum, as mentioned above.

The *antigen* must be prepared with a 0.9% NaCl solution and by 18°, the opacity of the suspension depending on this.

The reading depends entirely on the time allowed for the reaction, which, according to the first prescription, was 7 hours but has since been prolonged to 20 hours.

1. After *7 hours* the reading is difficult, takes much time, and is very tiresome and sometimes uncertain for the following reasons.

To decide whether a reaction is positive or negative requires

a lens in the case of a weak reaction. It is therefore necessary to examine all the tubes carefully with a lens, even the negatives.

In some, sera precipitation are found which resemble the specific precipitation, but these are often due to an infection which can be proved by incubation for a longer period.

The inhibition is often strongly pronounced; in some cases even total inhibition in all the 5 tubes, required by *Dreyer and Ward* for this test, is observed; the discovery that a strong positive W. R. has been observed in such sera has induced an increase of the number of the test-tubes to 12, which results in a strong positive reaction.

2. Since the time allowed for the reaction has been changed to *20 hours*, the reading must be considered very easy, because :

(*a*) It is possible to decide, without the use of a lens, whether the reaction is positive or negative, total precipitation often occurring even in weak reactions in the first tube;

(*b*) Precipitation occurring from infections can easily be distinguished from a real positive reaction;

(*c*) Inhibition zones occur much more rarely and are less pronounced.

In some cases, after 20 hours' incubation a very fine precipitation is observed corresponding to standard + (= 1.1 Σ unit); after leaving the tube 40 hours, this reaction makes no further progress and must then be considered negative; all such reactions have in our investigations been considered negative, our lowest limit for positive reactions being 1.5.

The calculation of the Σ unit is best undertaken on the basis of a precipitation which is not too strong, preferably from st. to tr.

Amendments.

For a large routine laboratory it might have some advantage to divide the Σ reaction into two tests : a qualitative and a quantitative; the qualitative test being then reduced to the use of two tubes only for each serum :

Tube N° 1, 20 drops of serum + 0 dr. NaCl + 6 dr. antigen (A. susp.).

Tube N° 2, 1 drop of serum + 19 dr. NaCl + 15 dr. antigen (B. susp.).

The serum is sucked in a dropping pipette, marked to take 21 drops; one drop is put into tube 2, the rest into tube 1; salt water and antigen are then added with a mechanical syringe. In the case of a positive reaction, the serum is then titrated quantitatively by means of nine tubes and in accordance with the usual method.

Remarks on the Quantitative Performance of the Test

Of the four reactions, only two are carried out quantitatively : the W. R. and Σ tests. These do not run at all on parallel lines, as

a W. R. of given strength may correspond to Σ reaction of a very varying amount of Σ units. Some examples are given in Table III. The W. R. units are indicated by a number, showing the reciprocal value of the smallest dose of the serum in question, which produces total inhibition of the hemolysis in a system with a total volume of 5 cc.

Table III.

Number of sera	W. R.	W. R. units	Σ units per cc.			
3	Negative	0	Resp.	4	22	45
3	40,100	1,5	»	4	20	48
3	0,20,100	5	»	10	50	200
3	0,0,20,100	10	»	45	200	400

The reason for this difference between the two reactions may be due either to the fact that they depend upon two different reagins, or that our present technique does not allow us to determine the exact strength of the reaction with sufficient regularity, or that other bodies or other circumstances interfere with the reaction.

To examine the exactitude with which it is possible to make the quantitative determination of the two tests, a series of syphilitic sera were divided into small portions, which were kept frozen and taken out for titration, one at a time, on successive days.

W. R.

Some examples are given in Table IV.

Table IV.

Serum No. 1 was tested	*W. R. units per cc.*
4 times to 20.100	2.5
9 » » 0.20.100	5
9 » » 0.10.100	7.5
25 » » 0. 0.20.100	10
16 » » 0. 0.10.100	15
2 » » 0. 0. 0.20.100	20
65 times	

Serum No. 2 was tested	*W. R. units per cc.*
Once to 0.0.20.100	10
» » 0.0.10.100	15
11 times » 0.0.0. 20.100	20
13 » » 0.0. 0. 10.100	30
24 » » 0.0. 0. 0. 40.100	40
3 » » 0.0. 0. 0. 10.100	80
43 times	

From this it appears that a serum (No. 1) was examined 65 times and showed variations from 2.5 to 20 W. R. units. Another serum (No. 2) showed variations from 10 to 80 W. R. units.

The examination of four syphilitic sera, titrated in all 200 times, showed the following results :

Table V.

85 times found		average value	
85	deviation	»	± 33 %
20	»	»	± 60 %
10	»	»	± 78 %

These great deviations are caused by the fact that different hemolytic systems often give a different complement fixation with the same serum, indicating a "strong" or a "weak" system. The fixing power of the system is dependent partly upon the value of the antigen, partly upon the faculty of the complement to be fixed in the complex : antigen-reagin-complement. It might be possible to compensate for the disturbances due to a "weak" resp. "strong" hemolytic system by determining the proportion between the strength (fixing power) of such a system and that of a "standard" system. If with a hemolytic system a control-serum showed for instance the double value of that found by the "standard" system, the value should be halved, whereas, if it showed half the value, it should then be doubled. This procedure is not, however, practicable, because a large number of experiments have proved that the *same* hemolytic system may sometimes act as a "strong" system for one serum, while for another serum, as a "normal" or even a "weak" one.

Sigma Test.

Table VI gives some examples of the variations which may be expected from the titration of different sera. In serum No. 16 the variations in 16 different titrations were from 45 to 50 Σ units; in serum No. 18, from 205 to 220 units.

Table VI.

Serum No. 16 was tested	Σ *units per cc.*
11 times to	45
4 » »	48.6
once »	50
16 times	
Serum No. 18 was tested	
13 times to	205
once »	220
14 times	

10 different syphilitic sera were examined, each titrated 9-11 times. All reactions were performed by the same antigen. It is not yet possible therefore to determine whether the different antigens would react differently with different sera in the Σ test.

With the 10 sera, 109 titrations were made :

In 95 cases, the value was quite similar;

In 14 cases, various values were observed, the greatest deviation not exceeding ± 6%.

From these results, it is obvious that the *quantitative W. R. test with the present technique presents a considerably greater error than the Σ test, which seems to furnish remarkably constant results.*

Conclusions

The Meinicke test, in spite of its advantage of being quite specific, is in its present form too slightly sensitive as compared with the other methods.

The Sachs-Georgi test gives quite useful results, the number of positive reactions being very near to that of the W. R. It presents a certain amount of reactions in cases without clinical signs of syphilis, and has the drawback that in its present form it does not work quantitatively.

The W. R. is quite specific and gives a high percentage of positive results; its drawback is its complexity and the difficulty sometimes met with in obtaining absolutely constant quantitative titration.

The Σ test is far the most sensitive of all reactions. Its technique is now simple and cheap. It allows a quantitative titration and affords the possibility of an international standardisation. It shows a certain number of positive reactions in cases without clinical signs of syphilis.

The W. R. and the Σ test appear to present the greatest advantage, and a further comparison between these two tests should be undertaken.

The sero-diagnosis of syphilis should be undertaken only by thoroughly trained workers and in adequately equipped laboratories.

PRELIMINARY REPORT

BY PROFESSOR GEORGE DREYER AND DR. H. K. WARD

Oxford.

In October 1921, the Medical Research Council, England, decided to carry out a comparison between the results obtained by the Wassermann and Sigma reactions for the diagnosis of syphilis. 895 serums were examined. The rules laid down in this comparison were as follows :

Each specimen of blood was divided into two parts, numbered, and forwarded respectively to the workers on the Wassermann and Sigma reactions. No information about the cases was available to either set of workers until the final result of the whole series had been forwarded by both sets of workers to the Medical Research Council. The Wassermann reaction was carried out in London by Professor J. McIntosh and Dr. P. Fildes according to the method laid down by them in *Medical Research Council Special Report* No. 14, save that, instead of using one quantity of serum, *viz.*, 0.1 cc., falling quantities of serum were used, *viz.* 0.1 cc., 0.05 cc., 0.025 cc., 0.0125 cc., 0.00625 cc. The result of the Wassermann reaction in each tube was expressed by the figures 4, 3, 2, 1, 0. Here, 4 means no hæmolysis, 3 means up to 25% hæmolysis, 2 means 50% hæmolysis, 1 means 75% and 0 means 100% hæmolysis. In this reaction, a reading of 4 in the highest concentration of serum, *i.e.*, 0.1 cc., is considered a positive reaction, while lower readings, *i.e.*, 3, 2 and 1, are only to be considered a positive reaction in known cases of syphilis. The Sigma reaction was carried out in Oxford by Professor Dreyer and Dr. H. K. Ward according to the method described by them in *The Lancet*, 1921, I, 956, save that the time of incubation was extended from 7 hours to 20 and 44 hours, as described in "An Additional Note on the Sigma Reaction" circulated among the workers under the League of Nations auspices. The results obtained by the Sigma reaction were expressed in standard Sigma units as laid down in the original communication. In this reaction, a reading of more than 1.5 units is considered a positive reaction, while a reading of 1.0 to 1.5 units is only to be considered a positive reaction in known cases of syphilis.

The following are the results obtained :

	Total	Wassermann R. positive	Sigma R. positive
Primary and Ex-Primary Syphilis. . .	110	60	67
Secondary and Ex-Secondary Syphilis.	252	155	191
Tertiary and Ex-Tertiary Syphilis. . .	124	107	118
Congenital Syphilis	32	25	29
Serological Syphilis	90	71	76
Controls	279	0	0
TOTAL	887	419	481

(NOTE. — By "Serological Syphilis" is meant those cases which, on the occasion of this test, or a previous test, have given a positive Wassermann reaction without other evidence of syphilis.)

(NOTE. — In the abbreviated table above, the relative number of untreated and treated cases is not indicated. The results of the two tests are therefore not for general comparison with the series of other workers unless the series contains the same proportion of untreated and treated cases.)

In the following six specimens (not included in above table), the Wassermann reaction was negative and the Sigma reaction was positive. In four specimens there was a clinical suspicion of syphilis, but no definite evidence, while in two specimens there was no clinical suspicion of syphilis. The cases are being further investigated. The details of these cases are :

Case	Date of specimen	Wa. R.	Sigma Units	Sex, Age	Diagnosis
1	1/VI. 1922	00000	2.8	M. 45	? Congenital Syphilis
2	8/VI. 1922	11000	7.0	F. 37	Iritis. Dislocation of lens
3	12/V. 1922 18/V. 1922	00000 00000	3.8 3.3	F. 58	Nasal sepsis (? syphilitic). No other signs suggestive of syphilis
4	16/III. 1922	00000	1.8	M. 28	Rheumatic fever
5	15/II. 1922	00000	1.8	M. 38	Syringo-myelia

In the following two specimens of serum (not included in the table), the Wassermann reaction was positive and the Sigma reaction negative. They are not included under the heading "Serological Syphilis", because, during the course of these tests, a certain amount of evidence was obtained that, occasionally, normal pregnant women develop a positive Wassermann reaction in the later months of pregnancy, the reaction becoming negative at the end of pregnancy and the child being born healthy with a negative Wassermann reaction. This question is being investigated further.

Case	Date of specimen	Wa. R.	Sigma Units	Sex, Age
1	13/III.22	44410	0	F. 23
	20/III.22	44210	0	

NOTE. — Pregnant 9 months. Wa. R. on 1/II.22 was doubtful, on 15/II.22 was doubtful, on 22/II.22 was positive. Treatment with Novarsenobillon was then started. Wa. R. was negative 17/V.22. The child was born 21/III.22, and at the age of 1 month was well and had a negative Wassermann reaction.

REPORT

BY N. GASIOROWSKI,

From the Lwow branch of the State Epidemiological Institute at Warsaw.

Concluding from the researches we have made up to now, we give the preference to the D. M. test comparatively to S. G., because of its easier technique and of the quantitative results it furnishes.

An important fact must not be omitted : we not infrequently observe in tests by the S. G. or D. M. methods a negative or + —results where B.W. is positive :

On	80	cases with	B.W.	+	S.G. gives with	antigen	W17—22	times; that is	27.50 %
»	748	»	»	—	»	»	L 1—95	» »	12,72 %
»	115	»	»	—	»	»	W33—17	» »	14.78 %
»	645	»	»	+	D. M.	»	L 2—234	» »	36.2 %
»	115	»	»	+	»	»	W34—17	» »	14.78 %

We observe also, although somewhat more rarely, that B.W. can be negative in cases where S. G. and D. M. are positive:

On	226	BW—	S.G	gives	with	antigen	W17— 0	times +,	that is 0 %
»	1,690	» —	»	»	»	»	L 1—71	» +,	» » 4.20 %
»	238	» —	»	»	»	»	W33—15	» +,	» » 6.30 %
»	1,426	» —	D. M.	»	»	»	L 2—17	» +,	» » 1.19 %
»	238	» —	»	»	»	»	W34— 9	» +,	» » 3.78 %

This discrepancy of results constitutes in most cases the weak point of these methods : the antigen being the principal cause thereof.

The difference between results obtained with different antigens is very great; we suppose therefore that, after having tried a greater number of antigens, we shall obtain a result such that differences between B.W., S. G. and D.M. will then disappear entirely or shall at least be reduced to the minimum.

We have followed till now 168 cases, which were examined repeatedly and still are in treatment in the hospitals.

The changes which can be detected in certain results of B.W.— also appear in S. G. and D. M. : we have nevertheless registered 5 cases of S. G. and 8 of D. M. which were discordant.

As a consequence of the absence of precise clinical data, we cannot establish precisely how far treatment and its intensity and duration have any influence on the diminishing or disappearance of positive reactions.

2,488 sera were examined from 22/III to 23/IX. 1922.

S.G. test with antigen.	W 17—	316
» »	L 1—	2,438
»	W 33—	356
TOTAL		3,107
The Meinicke test (D. M.) with antigen . . .	L 2—	2,071
» » » . . .	W 34—	353
TOTAL		2,424

The technique of the D. M. test is decidedly the simplest of all three (B. W., S. G., D. M.) because of the titration of its antigen. This titration is very easy, and its greatest advantage resides in its independence from the B. W. test.

Our point of view concerning group ± plays an important part in the appreciation and comparison of results of D. M. and S. G. with B. W.

In the S. G. test, the results, in agreement with B. W., are (considering + + +, + + and + as a unique group +) :

± as autonomous with ant. W17=91.14 %; with ant. L 1=87.86 %; with ant. W 33=82.44 %.
± as belonging to —W17=93.03 %; with ant. L 1=93.20 %; with ant. W 33=90.94 %.
± as belonging to +W17=94.62 %; with ant. L 1=90,98 %; with ant. W 33=86.12 %.
± eliminated, W17=95.98 %; with ant. L 1=95.92 %; with ant. W 33=93.73 %.

In the D. M. test, the results, in agreement with B. W., are :

± considered as autonomous,					with ant. L 2=79.97 %		with ant. W 34=88.39 %
±	»	belonging to	—	»	»	87.40 %	»	» 92.64 %
±	»	»	+	»	»	85.28 %	»	» 92.92 %
± eliminated,					»	91.86 %	»	» 96.83 %

This table shows great differences. The least exact results are obtained if we consider + as an autonomous group, and the best if we neglect the ±.

The results of the test are dependent from choice of antigen : the antigens W 17 and W 34 constantly give better results than the other antigens.

Even if we admit that the result of the investigation depends from the time spent between taking of blood and its testing, the cases examined up to now do not allow us to ascertain any notable differences.

In the S. G. and D. M. tests less fresh sera often give less differences with B. W. than fresh sera. The sera were tested from 1 to 18 days after their taking, but owing to the insufficiency of the elements of investigation, they cannot be used for drawing up of conclusions (see Table of Repeated Reactions).

Neither in comparing the reactions of feebly-hæmolytic and of strongly-hæmolytic sera in the B.W., S.G., and D.M. tests can great differences be detected (see table).

The principle object of our investigation was to compare the S.G. and D.W. tests with the B.W. reaction.

With this intention, we have made :

2,071 B.W. tests, and also with the same serum S.G. and D.M. with 1 antigen.
353 B.W. tests, and also with the same serum S.G. and D.M. with 2 antigens.

The following are the results :

I. 2,071 sera.

S.G. ant. L 1=87.16 %; D.M. ant. L 2=79.97 % (± as autonomous)
» 93.15 %; » 87.40 % (± as belonging t —)
» 90,30 %; » 5.28 % (± » +)
» 95.85 %; » 91.86 % (± eliminated)

II. 353 sera.

S.G. ant. L 1=87.54 %; ant. W 33=82.44 %; D.M. ant. 12=82.44 %; ant. W 34=88.39 %.
S.G. ant. L 1=92.64 %; ant. W 33=90.94 %; D.M. ant. 12=90.09 %; ant. W 34=92.64 %.
S.G. ant. L 1=90.66 %; ant. W 33=86.12 %; D.M. ant. 12= 87.54 %; ant. W 34=92.92 %.
S.G. ant. L 1=95.30 %; ant. W 33=93.73 %; D.M. ant. 12=94.64 %; ant. W 34=96.83 %.

Comparing the groups I and II, we see that D.M. with antigen W 34 gives best results. S.G. comes next with antigen L 1.

If the groups + — are eliminated, the agreement of tests attains 96.83 — 95.85 %. It must also be borne in mind that coincidence of results with 4 antigens appears in 16 cases for S.G. and D.M., whereas differences show themselves for B.W. (ant. L 1 — 26, L 2 — 35, W 33 — 32, W 34 — 26).

We may ascertain that the agreement between the flocculation tests and B. W. depends alone on the choice of antigen.

Table of Repeated Reactions.

from March 30th to August 13th, 1922

No.	No. of protoc.	Diagnosis	Date	B. W.	Sachs-Georgi antigen		D. M. antigen	
					L 1	W 33	L 2	W 34
1	1915	Lat. syphilis	9/4	—	—			
	3826		6/7	+++	+++		+++	
2	1720	Lat. syphilis	30/3	+++	++			
	4012		13/7	—	—		—	
3	1746	Syph. II	30/3	+++	+++			
	3872		6/7	—	—		—	
4	1995	Lat. syphilis	13/4	—	—			
	4552		6/8	+++	+		++—	
5	2007	Lat. syphilis	13/4	—	—			
	3144		8/6	—	—		—	
	5430		21/9	—	—	+—	—	—
6	2049	Lat. syphilis	20/4	—	—			
	5411		21/9	—	—	—	.—	—
7	2081	Lat. syphilis	24/4	+++	++			
	3359		17/6	+++	—		—	
	4896		26/8	—	—	—	—	—
8	2087	Lat. syphilis	23/4	—	—			
	4466		3/8	—	—		—	
9	2336	Lat. syphilis	7/5	—	—		—	
	2622		21/5	—	—		—	
10	2350	Lat. syphilis	7/5	+++	+—		—	
	4325		27/7	—	—			
11	2361	Syphilis ?	7/5	—	—		—	
	2799		25/5	—	+		+—	
	3140		8/6	—	—		—	
12	2375	Lat. syphilis	7/5	+++	+++		+—	
	2797		25/5	+++	+++		+	
	4487		3/8	+++	+++		+	
13	2444	Syphilis II	11/5	—	—		—	
	4198		20/7	—	—		—	
14	2447	Lat. syphilis	10/5	—	—		—	
	3653		28/6	—	—		—	
15	2450	Lat. syphilis	15/5	+++	+++		+++	
	3374		17/6	+++	+		+—	
	3929		7/7	—	—		—	
16	2533	Lat. syphilis	17/5	—	+		—	
	3118		7/6	—				
	3439		21/6	+—	—		+—	
17	2534	Lat. syphilis	17/5	+	+		—	
	3438		21/6	+++	+		+—	

No.	No. of protoc.	Diagnosis	Date	B. W.	Sachs-Georgi antigen		D. M. antigen	
					H. 1	W 33	L 2	W 34
18	2534	Lat. syphilis	17/5	—	—		—	
	3007		31/5	—	—		—	
19	2538	Relapsing pap.	17/5	—	—		—	
	3113	syph.	7/6	—	—		—	
20	2539	Lat. syphilis	17/5	—	—		—	
	3797		5/7	—	—		—	
21	2540	Lat. syphilis	18/5	+—	±		—	
	2953		1/6	—	±—		+—	
22	2541	Lat. syphilis	17/5	—	—		—	
	2608	provocatio	»	—	—		—	
23	2543	Lat. syphilis	17/5	—	—		—	
	2609	provocatio	»	—	—		—	
24	2544	Lat. syphilis	17/5	+++	+		—	
	4973		30/8	±±±	+	±—	+—	+
25	2545	Relaps. mac.	17/5	+++	+		+	
	3274	pap. syph.	14/6	±±	—		—	
	4098		15/7	—	—		—	
	5060		2/9	—	—	—	—	—
	5359		16/9	—	—	—	—	—
26	2554	Lat. syphilis	18/5	—	—		—	
	4337		27/7	—	—		—	
27	2555	Lat. syphilis	18/5	—	—		—	
	3842		6/7	—	—		—	
28	2562	Lat. syphilis	18/5	—	—		—	
	3842		6/7				+—	
29	2565	Lat. syphilis	18/5	—	—		—	
	3646		29/6	+++	±±±		+++	
30	2566	Lat. syphilis	18/5	—	—		—	
	3855		6/7	—	—		—	
31	2568	Lat. syphilis	18/5	+++	+++		++	
	3841		6/7	+++	+++		±±±	
32	2570	Lat. syphilis	18/5	+	±—		+—	
	4338		27/7	—	±—		—	
33	2625	Lat. syphilis	21/5	+++	±±±		+	
	3380		18/6	±±±	+		±—	
34	2629	Syphilis II	21/5	+++	+++		±±±	
	3582		26/6	+++	—±		+	
	3753		2/7	±±±	—±		+	
	3800		6/7	—	±—		—	
	4190		20/7	—	+—		—	
35	2630	Syphilis I	21/5	±±±	+++		++	
	3748		2/7	+	—		+	

No.	No. of protoc.	Diagnosis	Date	B. W.	Sachs-Georgi antigen		D. M. antigen	
					L 1	W 33	L 2	W 31
36	2647	Lat. syphilis	20/5	—	—		—	
	4193		19/7	—	—		—	
	4966		30/8	—	—	—	+—	—
37	2648	Lat. syphilis	20/5	—	—		—	
	3217		10/6	—	—		—	
	3437		21/6	—	—		—	
38	2649	Lat. syphilis	20/5	—	+++		+—	
	2724		24/5	—	+++		—	
39	2650	Lat. syphilis	20/5	—	—		—	
	3214		10/6	—	—		+—	
	3447		21/6	—	—		+—	
40	2652	Lat. syphilis	20/5	—	—		—	
	2686	provocatio	»	—	—		—	
41	2653	Papul. syphilis	21/5	++	++		—	
	2935		1/6	++	++		—	
42	2655	Relap. pap.	20/5	—	—		—	
	3212	syphilis	10/6	+++	++		+—	
43	2656	Relap. pap.	20/5	+++	++		+	
	3710	syphilis	2/7	+	+		+	
	4376		29/7	+	+		+	
44	2719	Lat. syphilis	24/5	++	+		+—	
	3267		14/6	+++	+		—	
45	2720	Lat. syphilis	24/5	+—	—		—	
	3440		21/6	+++	+—		+	
	4375		29/7	+++	++		+	
46	2721	Lat. syphilis	24/5	—	—		—	
	3004		31/5	—	—		—	
47	2722	Relap. pap.	24/5	+++	—		—	
	4806	syphilis	19/8	+++	—	—	—	—
48	2723	Relap. syphilis	24/5	+++	+++		—	
	3271		14/6	++	+		+—	
	3893		8/7	—	—		—	
49	2725	Observatio	24/5	—	—		—	
	2926		31/5	—	—		—	
	2943	provocatio	»	—	—		—	
50	2726	Syph. of vessels	24/5	+++	+++	—	++	
	4838		23/8	+++	++	+	++	+
51	2727	Lat. syphilis	24/5	—	—		—	
	3707		2/7	—	—		—	
52	2730	Lat. syphilis	24/5	—	—		—	
	2814	provocatio	»	—	—		—	
53	2737		25/5	+++	+++		+++	
	3823	Syph. II	6/7	++	+++		+	
54	2739	Syph. II	25/5	+++	++		+	
	3815		6/7	+—	—		—	

No.	No. of protoc.	Diagnosis	Date	B. W.	Sachs-Georgi antigen		D. M. antigen	
					L 1	W 33	L 2	W 34
55	2745	Syph. I	25 5	−	−		−	
	3827		6/7	−	−		−	
56	2761	Lat. syphilis	24/5	−	−		−	
	4442		2/8	−	−		−	
	3068		2/9	−	−	−	+−	+−
57	2800	Lat. syphilis	25/5	+++	++		+	
	3505		21/6	+++	+		+−	
58	2801	Lat. syphilis	24/5	+++	+++		+++	
	3336		14/6	−	++		+	
	3642		28/6	+++	+++		+	
59	2802	Lat. syphilis	25/5	−	−		+−	
	3507		21/6	−	−		−	
60	2815	Lat. syphilis	24/5	−	−		−	
	3006		31/5	−	−		−	
61	2817	Relaps. pap. syp.	24/5	+++	+++		++	
	4423		2/8	+++	+++		−	
62	2855	Mac. pap. syph.	27/5	+	+		+−	
	3400		17 6	+	−		−	
	5065		2/9	+++	+++	+++	++	++
63	2861	On observation	27/5	−	−		−	
	3156		3/6	+−	−		+−	
	4930		26/8	−	−	−	−	−
64	2863	Mac. pap. syph.	27/5	+−	−		−	
	3283		14/6	+−	−		−	
65	2864	Pap. pust. syph.	27/5	++	++		+	
	3469		21/6	+++	+		+−	
	4816		19/8	−	+−	+−	+−	−
66	2895	Lat. syphilis	31/5	+−	+		−	
	3376		17/6	+	+−		+−	
	3746		2/7	−	−		−	
67	2924	Lat. syphilis	31/5	−	−		−	
	2932	provocatio	»	−	−		−	
68	2925	Lat. syphilis	31/5	−	−		−	
	3060		3/6	−	−		−	
	3117		7/6	−	−		−	
	3619		28/6	−	−		−	
69	2927	Init. ulcus	31/5	−	+−		+−	
	4185		19/7	−	−		−	
70	2929	Lat. syphilis	31/5	+−	+++		+−	
	3156		7/6	+−	+++		+	
	3899		8/7	+	++		+−	
71	2930	Init. ulcus	31/5	−	−		−	
	3005	provocatio?	»	−	−		−	
	3264		14/6	−	−		−	
72	2931	Init. sclerosis	31/5	+++	+++		++	
	3436		21/6	+++	+++		++	
	3898		8/7	+++				

No.	No. of protoc.	Diagnosis	Date	B. W.	Sachs-Georgi antigen		D. M. antigen	
					L 1	W 33	L 2	W 34
73	2934	Relapsing syph.	31/5	−	−		−	
	3783		5/7	−	−		−	
74	2935	Lat. syphilis	31/5	++	++		−	
	4437		2/8	+++	++		+−	
75	2937	Lat. syphilis	31/5	+++	+++		−	
	3979		12/7	+−	+−		−	
	4436		2/8	+−	+−		−	
76	2938	?	31/5	−	−		−	
	3265		14/6	−	−		−	
	3709		2/7	−	−		−	
	4238		22/7	−	−		−	
77	2939	Lat. syphilis	31/5	−	−		−	
	4428		2/8	+++	+++		+	
78	2940	Lat. syphilis	31/5	−	−		−	
	3154		7/6	−	−		−	
79	2941	Relapsing pap. syphilis	31/5	+++	+++		++	
	3711		2/7	+	+		+	
	4431		2/8	−	−		−	
80	2946	Mac. pap. syph.	31/5	+++	+++		+++	
	3284		14/6	+++	+++		+	
	4105		15/7	+−	−		−	
	4292		26/7	+−	+−		−	
81	2947	Papul. syphilis	31/5	+++	+++		−	
	3278		14/6	+++	+++		+−	
	3721		2/7	++	++		−	
82	2948	Relaps. pap. syphilis	31/5	+++	+		+++	
	3277		14/6	+++	+		+++	
	4104		15/7	+++	+++		+	
	4293		26/7	+++	+++		++	
	4448		2/8	+++	+++		++	
83	2972	Lat. syphilis	1/6	+++	+++		−	
	3500		21/6	+++	−		−	
84	2975	Syph. I	1/6	−	−		−	
	3886		6/7	−	−		−	
85	2988	Lat. syphilis	1/6	−	−		−	
	4491		3/8	−	−		−	
86	3003	Lat. syphilis	1/6	−	−		−	
	5362		17/9	−	−	−	−	−
87	3053	Relap. mac. pap. syphilis	3/6	+++	++		+	
	4189		19/7	−	−		−	
88	3054	Lat. syphilis	3/6	+−	−		−	
	3800		5/7	−	+−		−	
	4190		19/7	−	+−		−	
89	3058	Lat. syphilis	3/6	+	++		+	
	4240		22/7	+−	+		−	
	5347		16/8	+	+	++	+	++

No.	No. of protoc.	Diagnosis	Date	B. W.	Sachs-Georgi antigen		D. M. antigen	
					I. 1	W 33	I. 2	W 34
90	3068	On observation	26/4	−	−			
	4203		20/7	−	−		−	
	5275		14/9	−	−	−	−	−
91	3089	Lat. syphilis	30/4	+++	+−			
	2452		14/5	+−	−		−	
92	3094	Lat. syphilis	7/6	−	−		−	
	3986		13/7	−	−		−	
93	3114	Rel. syphilis	7/6	−	−		−	
	4099		15/7	−	−		−	
94	3116	Lat. Syphilis	7/6	−	+		+−	
	3155	provocatio	»	−	+		−	
95	3119	Lat. syphilis	7/6	−	+		+	
	3223		10/6	+	++		−	
	5223		13/9	+++	+++	+++	+++	+++
96	3120	Lat. syphilis	7/6	−	−		−	
	3790		5/7	−	−		−	
97	3125	Rel. mac. pap.	7/6	+++	+++		++	
	3876	syphilis	5/7	+++	++		+	
	4280		26/7	+	+		−	
98	3171	Lat. syphilis	11/6	+++	+		+−	
	3583		26/6	++	+		−	
	3932		9/7	−	−		−	
99	3174	Syph. II	10/6	+++	+++		++	
	3580		24/6	+++	+++		+++	
	4133		14/7	+	+		−	
100	3190	Lat. syphilis	11/6	−	−		+−	
	4462		3/8	+−	+−		−	
101	3207	Lat. syphilis	10/6	−	−		−	
	3221	provocatio	»	−	−		−	
102	3209	?	10/6	+++	+++		+	
	3896		8/7	+	+++		+	
103	3215	Syph. of	10/6	−	−		−	
	5164	nervous syst.	9/9	−	−	−	+−	−
104	3216	Lat. syphilis	10/6	−	−		−	
	5184		9/9	−	−	−	+−	−
105	3220	Pap. pust. syph.	10/6	+++	+++		+++	
	4106		15/7	+++	++		−	
	4293		26/7	+	++		+−	
	4447		2/8	−	+−		−	
106	3263	Lat. syphilis	14/6	−	−		−	
	4543		5/8	−	−		−	
	4625		9/8	−	−		−	
107	3266	Lat. syphilis	14/6	−	−		−	
	4093		15/7	−	−		−	

No.	No. of protoc.	Diagnosis	Date	B. W.	Sachs-Georgi antigen		D. M. antigen	
					L 1	W 33	L 2	W 34
108	3268	Lat. syphilis	14/6	+++	+		−	
	4092		15/7	+−	−		−	
	5230		13/9	+−	+−	+	+−	+−
109	3279	On observation	14/6	+++	+++		−	
	4929		26/8	+	+	+	+	+
110	3279	Pap. syphilis	14/6	+++	+++		+−	
	3726		2/7	+−	+		−	
111	3282	Pap. syphilis	14/6	+++	+		−	
	3722		2/7	+−	−		+−	
	4103		15/7	−	−		−	
112	3318	Lat. syphilis	15/7	−	−		−	
	4332		27/7	−	−		−	
113	3331	Syph. II	14/6	+++	+		−	
	3645		28/6	+−	+−		+−	
114	3333	Lat. syphilis	15/6	+++	++		+	
	3493		21/6	+++	+		+++	
115	3397	Lat. syphilis	17/6	+++	+		−	
	3717		2/7	++	+		+	
116	3402	Lat. syphilis	17/6	+++	+++		+	
	4097		15/7	+	++		−	
	5357		16/9	+++	+	+	+	++
117	3448	Relap. pap. syp.	21/6	−	−		−	
	5358		16/9	−	−	−	−	−
118	3452	Lat. syphilis	21/6	−	−		−	
	4382		29/7	−	−		−	
119	3461	Papul. syphilis	21/6	+++	+		+	
	3725		2/7	+−	+		+	
120	3463	Papul. syphilis	21/6	+++	+++		++	
	3724		2/7	+++	+++		++	
121	3465	Pap. syphilis	21/6	+++	+++		+++	
	4815		19/7	−	−	−	−	−
122	3466	Pap. syphilis	21/6	+++	+++		+++	
	3728		2/7	+++	+++		+++	
	4102		15/7	+++	+++		+	
	4814		19/8	+++	++	++	−	+−
	4931		26/8	+++	++	++	+	+
123	3509	Lat. syphilis	21/6	+++	+−		−	
	4199		20/7	+	+−		−	
124	3538	Rel. pap. syph.	24/6	+++	++		+−	
	4094		15/7	+−	+−		−	
	5225		13/9	−	−	−	−	−
125	3539	Lat. syphilis	24/6	−	−		−	
	4191		19/7	−	−		−	

No.	No. of protoc.	Diagnosis	Date	B. W.	Sachs-Georgi antigen		D. M. antigen	
					L 1	W 33	L 2	W J4
126	3540	Lat. syphilis	24/6	+−	−		−	
	3617		28/6	−	−		−	
	3716		2/7	−	−		+−	
127	3541	Syphilitic gums	24/6	+++	+++		+++	
	3715		2/7	+++	+++		+++	
	4184		19/7	+++	+++		+++	
128	3543	Lat. syphilis	24/6	−	−		−	
	4928		26/8	−	−	−	−	−
129	3616	Lat. syphilis	28/6	−	−		−	
	3620	provocatio	»	−	−		−	
130	3621	Lat. syphilis	28/6	+−	−		−	
	3804		5/7	−	−+		−	
131	3623	Lat. syphilis	28/6	−	−		−	
	4837		23/8	−	−	+−	−	−
132	3652	Lat. syphilis	29/6	+	+		+	
	4329		27/7	−	−		−	
133	3676	Optic neuritis	28/6	+++	+++		+++	
	4384		29/7	+++	+		+++	
134	3706	Lat. syphilis	2/7	+−	+−		−	
	4237		22/7	−	+−		−	
135	3712	Rel. mac. pap.	2/7	+++	+		+	
	4279	syphilis	26/7	−	−		−	
136	3713	Rel. pap. syph.	2/7	++	++		+	
	4633		9/8	−	+−		−	
137	3719	Lat. syphilis	2/7	−	−		−	
	3891		8/7	−	−		−	
138	3741	Lat. syphilis	2/7	++	++		+	
	4440		2/8	++	++		+−	
139	3782	Lat. syphilis	5/7	−	−		−	
	5224		13/9	−				
	5397		20/9	−	−	−	−	−
140	3785	Lat. syphilis	5/7	−	−		−	
	4627		9/8	−	−		−	
141	3786	Lat. syphilis	5/7	+	−		−	
	4545		5/8	+	+−		−	
142	3791	Relap. pap. syp.	5/7	+++	+++		+	
	4282		26/7	++	++		−	
	5220		13/9	−	−	−	−	−
143	3792	Lat. syphilis	5/7	+	+−		−	
	4381		29/7	+	+		−	
144	3793	Lat. syphilis	5/7	+	+		+	
	4239		22/7	+−	+		−	
145	3794	Lat. syphilis	5/7	−	−		−	
	5349		16/9	−	−	−	−	−

No.	No. of protoc.	Diagnosis.	Date	B. W.	Sachs-Georgi antigen		D. M. antigen	
					L 1	W 33	L 2	W 34
146	3796	On observation	5/7	−	−		−	
	4095		15/7	−	−		−	
	4192		19/7	−	−		−	
147	3803	Macul. pap.	5/7	+++	++		+	
	4373	syphilis	29/7	+++	+		+	
148	3882	?	5/7	+−	++		−	
	4542		5/8	++	++		−	
149	3891	Lat. syphilis	8/7	+−				
	4096		15/7	+	+−		−	
150	3392	Lat. syphilis	8/7	+	+		+−	
	4626		9/8	−	+−		+−	
	5350		16/9	++	++	+++	++	++
151	3895	Lat. syphilis	8/7	−				
	3976		12/7	−	−		−	
152	3897	Lat. syphilis	8/7	−	−		−	
	4101		15/7	−	−		−	
153	3971	Nervous syph.	12/7	+−	+	+	−	
	5352		16/9	+−	+		+−	+−
154	3975	Rel. papul. syph	12/7	+−	++	+	−	
	5227		13/9	+−	+−		+	+
155	3980	Lat. syphilis	12/7	−	−		−	
	4188		19/7	−	−		−	
156	3981	Lat. syphilis	12/7	−	−		−	
	4194		19/7	−	−		−	
157	4012	Lat. syphilis	13/7	−	−	+−	−	
	5420		21/9	+	−		+−	+−
158	4079	Lat. syphilis	16/7	−	−		−	
	4468		3/8	−	−		−	
159	4088	On observation	15/7	+++	+++		+−	
	4632		5/8	−	+−		−	
160	4089	Lat. syphilis	15/7	−	−		−	
	4372		29/7	−	+−		−	
161	4091	Lat. syphilis	15/7	−	−		−	
	4236		22/7	−	−		−	
162	4100	Rel. papul. syph	15/7	−	−	−	−	
	5354		16/9	−	−		−	−
163	4131	Syph. II	16/7	+++	+++		+	
	4268		23/7	+++	+++		+−	
	4410		30/7	+++	+++		++	
164	4358	Rel. pap. Syph.	26/7	−	+−		−	
	5222		13/9	+−	−	+	−	−

No.	No. of protoc.	Diagnosis	Date	B. W.	Sachs-Georgi antigen		D. M. antigen	
					L 1	W 33	L 2	W 34
165	4371	On observation	29/7	—	—		—	
	4548		5/8	—	—		—	
166	4438	Lat. syphilis	2/8	—	+		—	
	5417		20/9	—	+—	+	+—	+—
167	4672	Syph. II	13/8	—	—	—	—	—
	5337		17/9	—	—	+—	—	—
168	4715	Syphilis II	13/8	+++	+++	+++	+	+
	5389		21/9	—	—	—	—	—

Date of blood-taking and result of investigation from May 6th to September 23rd, 1922.

Reactions made with antigen		Days gone since the blood was taken	Results in disagreement with B. W. tests											
			Sachs-Georgi with antigen						D. M. with antigen					
			L 1			W 33			L 2			W 34		
L 1 L 2	W 33 W 34		+	—	Total %	+	—	Total %	+	—	Total %	+	—	Total %
900		I	31	26	6.33				27	94	13.44			
281	12	II	16	7	8.18				4	17	7.47			
133	58	III	4	4	6.01	2	1	5.17	2	16	13.53	2	2	6.89
211	92	IV	6	7	6.16	5	6	9.24		21	9.95	3	4	5.88
72	49	V	1	5	8.33	2	3	10.20	1	11	16.52		3	6.12
37	5	VI	2	1	8.10	1		20.00	2	4	16.21			
34	6	VII								1	2.94			
13	10	VIII		1	7.69	1	1	20.00		2	15.38		1	10.00
23	20	IX		2	8.68		2	10.00		3	13.04		2	10.00
61	2	X	1	1	3.27		1	50.00		5	8.19			
140		XI	5	2	5.00					12	18.57			
11	10	XII	2		18.18	3		30.00	2		18.18	4		40.00
17	17	XIII	1		5.88	1		5.88		2	11.76		1	5.88
14	14	XIV												
84	9	XV		1	1.19				1	13	16.66			
29	21	XVI		1	3.44		2	9.90	1	4	17.24			
2		XVII												
9	1	XVIII								1	11.11			

Influence of Hæmolytic Serum on Reaction, as established by investigations between March 22nd and September 23rd, 1922.

Slightly hæmolytic sera :

B. W.	Quantity	Sachs-Georgi			Meinicke			
		+	+—	—	+	+—	—	
+	60	60	2	4	54	7	5	Sachs-Georgi antigen L 1
+—	4		3	1		4		DM. » L 2
—	144	2	3	139	3	5	136	
TOTAL	214	62	8	144	57	16	141	

If we consider +++, ++ and + as a unique group +:

Results in agreement with BW given by S. G. in 94.34%, by DM in 90.67%.

Results in agreement if ± considered as — by S. G. in 96.27%, by DM in 93.00%.

Results in agreement if ± considered as + by S. G. in 95.33%, by DM in 93.93%.

Results in agreement if ± be neglected by S. G. in 97.08%, by DM in 95.96%.

Strongly hæmolytic sera :

B. W.	Quantity	Sachs-Georgi			Meinicke			
		+	+—	—	+	+—	—	
+	18	14	2	2	13	2	3	Sachs-Georgi antigen L 1
+—	5	1	1	3		1	4	DM. » L 2
—	33	2	1	30	1	1	31	
TOTAL	56	17	4	35	14	4	38	

If we consider +++, ++ and + as a unique group + :

Results in agreement with BW given by S. G. : in 80.36%, by DM in 80.36%.

Results in agreement if ± considered as — : by S. G. in 87.50%, by DM in 89.29%.

Results in agreement if ± considered as + : by S. G. in 85.72%, by DM in 83.93%.

Results in agreement if ± be neglected : by S. G. in 91.50%, by DM in 91.50%.

S.G. Antigen W 17 **Protocol No. I.** 9/IV 3/V 1922

	TREATED							UNTREATED						
Wassermann	Number	Sachs-Georgi					Meinicke	Number	Sachs-Georgi					Meinicke
		+++	++	+	±	−			+++	++	+	±	−	
Syphilis I														
+++	5		1	3	1									
++	1					1								
+	1				1			2				1	1	
±														
−	1					1		6					6	
Total .	8		1	3	2	2		8				1	7	
Syphilis II														
+++	15	5	3	5	2									
++	1	1												
+														
±	1					1								
−	7					7								
Total .	24	6	3	5	2	8								
Syphilis III														
+++														
++														
+	1				1									
±														
−														
Total .	1				1									
Latent Syphilis														
+++	41	11	11	13	4	2								
++	2			1		1								
+	5		1			4								
±	4				2	2								
−	97				1	96								
Total .	149	11	12	14	7	105								

PARASYPHILIS

TREATED								UNTREATED						
Wasser-man	Number	Sachs-Georgi					Meinicke	Number	Sachs-Georgi					Meinicke
		+++	++	+	±	—			+++	++	+	±	—	
+++														
++														
+														
±														
—	2					2								
TOTAL .	2					2								

DOUBTFUL						WITHOUT ANAMNESIS						
+++	12	5	1	5	1							
++	2	1			1							
+	2			1	1							
±	2				2							
—	72				72							
TOTAL .	90	6	1	6	77							

NOT SYPHILITIC						TOTAL					
+++						73	21	16	26	7	3
++						6	2		1		3
+						11		1	1	3	6
±						7				2	5
—	34				34	219				1	218
TOTAL .	34				34	316	23	17	28	13	235

If we consider +++, ++ and + as a unique group + :

S. G. ant. W 17 gives results in agreement with B. W. — : 91.14%.

S. G. ant. W 17 gives results in agreement if ± be regarded as — : 93.03%.

S. G. ant. W 17 gives results in agreement if ± be regarded as + : 94.62%.

S. G. ant. W 17 gives results in agreement if ± be neglected : 95.98%.

S.G. antigen L. 1 **Protocol No. II.** 22/III 23/IX 1922

	TREATED							UNTREATED						
Wassermann	Number	Sachs-Georgi					Meinicke	Number	Sachs-Georgi					Meinicke
		+++	++	+	±	−			+++	++	+	±	−	
Syphilis I														
+++	20	8	3	6	3			8	4	2	2			
++	3		1	1		1								
+	6				1	5		5			1	1	3	
±	1					1		5				1	4	
−	28			1	1	26		14				2	12	
Total .	58	8	4	8	5	33		32	4	2	3	4	19	
Syphilis II														
+++	121	66	26	25	1	3		17	13	3	1			
++	20	4	9	6		1								
+	11	1	1	7	2			2					2	
−	20		3	5	5	7								
±	78	1	1	1	15	60		4					4	
Total .	250	72	40	44	23	71		23	13	3	1		6	
Syphilis III														
+++	9	6	1	2				2	1		1			
++	3	1	1	1										
+	1				1									
±														
−	12					12		1					1	
Total .	25	7	2	3	1	12		3	1		1		1	
Latent Syphilis														
+++	212	104	43	42	9	14		6	2	2	2			
++	34	3	7	17	2	5		1		1				
+	65	4	12	29	8	12								
±	61	2	4	16	21	18		1					1	
−	678	1		23	47	607		7					7	
Total .	1,050	114	66	127	87	656		15	2	3	2		8	

PARASYPHILIS

TREATED								UNTREATED						
Wassermann	Number	Sachs-Georgi					Meinicke	Number	Sachs-Georgi					Meinicke
		+++	++	+	±	−			+++	++	+	±	−	
+++	10	5	4	1				2	2					
++	2		1	1										
+	4		2	2										
±	4			2		2								
−	14			1	2	11		1					1	
TOTAL .	34	5	7	7	2	13		3	2				1	

DOUBTFUL							NO ANAMNESIS						
+++	130	66	27	27	4	6							
++	24	6	4	7	3	4							
+	32	3	6	18	5								
±	28	1	1	2	5	19							
−	596		1	4	12	579							
TOTAL .	810	76	39	58	29	608							

NOT SYPHILITIC							TOTAL					
+++							537	277	111	109	17	23
++							87	14	24	33	5	11
+							124	8	21	56	18	21
±							116	3	8	25	31	49
−	137			1	1	135	1,574	2	2	31	81	1458
TOTAL .	137			1	1	135	2,438	304	166	254	152	1562

If we consider +++, ++ and + as a unique group + :

S. G. ant. L. 1 gives results in agreement with B. W. 87.86%.

S. G. ant. L. 1 gives results in agreement if ± be considered as — 93.20%.

S. G. and L. 1 gives results in agreement if ± be considered as + 90.98%.

S. G. ant. L. 1 gives results in agreement if ± be neglected 95.92%.

Antigen S.G. L 1
» D.M. L 2

Protocol No. III.

6/V — 23/IX 1922

Wassermann	TREATED Number	Sachs-Georgi +++	++	+	±	−	Meinicke +++	++	+	±	−	UNTREATED Number	Sachs-Georgi +++	++	+	±	−	Meinicke +++	++	+	±	−
SYPHILIS I																						
+++	15	7	3	3	2		1	3	4	2	5	8	4	2	2			2	2	**2**		2
++	2		1	1					1		1											
+	3					3			2		1	3			1		2				1	2
±	1					1					1	1					1					1
−	24			1	1	22				1	23	12				3	9			1	2	9
TOTAL . . .	45	7	4	5	3	26	1	3	7	3	31	24	4	2	3	3	12	2	2	3	3	14
SYPHILIS II																						
+++	110	62	24	21		3	33	15	39	13	10	17	13	3	1			6	8	2	1	
++	19	3	9	6		1			11	2	6											
+	11	1	1	7	2				3	4	4	2					2					2
±	20		3	5	5	7			1	5	14											
−	74	1	1	1	15	56			2	8	64	4					4					4
TOTAL . . .	234	67	38	40	22	67	33	15	56	32	98	23	13	3	1		6	6	8	2	1	6

Wassermann	TREATED Number	Sachs-Georgi +++	Sachs-Georgi ++	Sachs-Georgi +	Sachs-Georgi ±	Sachs-Georgi −	Meinicke +++	Meinicke ++	Meinicke +	Meinicke ±	Meinicke +	UNTREATED Number	Sachs-Georgi +++	Sachs-Georgi ++	Sachs-Georgi +	Sachs-Georgi ±	Sachs-Georgi −	Meinicke +++	Meinicke ++	Meinicke +	Meinicke ±	Meinicke −
Syphilis III																						
+++	9	6	1	2			6	1	2			2	1		1			1		1		
++	3	1	1	1				1		1	1											
+																						
±																						
−	12					12					12	1				1						1
Total. . . .	24	7	2	3		12	6	2	2	1	13	3	1		1	1		1		1		1
Latent Syphilis																						
+++	172	90	31	31	7	13	39	16	63	22	32	6	2	2	2			1		3	2	
++	32	3	7	16	2	4		2	6	7	17	1		1								1
+	60	4	11	29	8	8			17	14	29											
±	59	2	4	16	20	17		1	3	16	93	1				1						1
−	588	1		23	47	517		1	5	37	545	7				7					1	6
Total. . . .	911	100	53	115	84	559	39	20	94	96	662	15	2	3	2	8		1		3	3	8

Protocol No. III (*continued*).

Wassermann	TREATED Number	Sachs-Georgi +++	Sachs-Georgi ++	Sachs-Georgi +	Sachs-Georgi ±	Sachs-Georgi −	Meinicke +++	Meinicke ++	Meinicke +	Meinicke ±	Meinicke −	UNTREATED Number	Sachs-Georgi +++	Sachs-Georgi ++	Sachs-Georgi +	Sachs-Georgi ±	Sachs-Georgi −	Meinicke +++	Meinicke ++	Meinicke +	Meinicke ±	Meinicke −
PARASYPHILIS																						
+++	9	5	4				3	2	4			2	2					2				
++	1		1								1											
+	4		2	2					3		1											
±	4			2		2				1	3											
−	12			1	2	9					12	1					1					1
TOTAL. . . .	30	5	7	5	2	11	3	2	7	1	17	3	2				1	2				1
	DOUBTFUL											WITHOUT ANAMNESIS										
+++	108	55	24	22	2	5	18	23	38	17	12											
++	19	5	4	6	2	2			8	6	5											
+	29	3	6	15	5		1		7	12	9											
±	21		1	2	4	14				1	20											
−	481		1	4	12	464			3	22	456											
TOTAL. . . .	658	63	36	49	25	485	19	23	56	58	502											

NOT SYPHILITIC												TOTAL										
Wassermann	Number	Sachs-Georgi					Meinicke					Number	Sachs-Georgi					Meinicke				
		+++	++	+	±	−	+++	++	+	±	−		+++	++	+	±	−	+++	++	+	±	−
		NOT SYPHILITIC											TOTAL									
+++												458	247	91	85	11	21	111	71	157	58	61
++												77	12	24	30	4	7		3	26	16	32
+												110	8	20	53	15	14	1		32	31	46
±												107	2	8	25	29	43		1	4	23	79
−	103			1	1	101				4	99	1,319	2	2	31	81	1,203		1	11	75	1,232
TOTAL. . .	103			1	1	101				4	99	2,071	271	148	224	140	1,288	112	76	230	203	1,450

If we consider +++, ++ and + as a unique group + :

S. G. with ant. L. 1 gives results in agreement with B. W. 87.16%
» » » » if ± be considered as − 93.15%
» » » » if ± be considered as + ... 90.30%
» » » » if ± be neglected 95.85%

D. M. with ant. L. 2 gives results in agreement with S. G. 79.97%
» » » » if ± considered as − 87.40%
» » » » if ± considered as + ... 85.28%
» » » » if ± be neglected 91.86%

Antigen S.G. L 1.
Antigen D.M. L 2.

Protocol No. IV. 23/VIII—23/IX 1922.

Wassermann	TREATED Number	Sachs-Georgi +++	Sachs-Georgi ++	Sachs-Georgi +	Sachs-Georgi ±	Sachs-Georgi −	Meinicke +++	Meinicke ++	Meinicke +	Meinicke ±	Meinicke −	UNTREATED Number	Sachs-Georgi +++	Sachs-Georgi ++	Sachs-Georgi +	Sachs-Georgi ±	Sachs-Georgi −	Meinicke +++	Meinicke ++	Meinicke +	Meinicke ±	Meinicke −
SYPHILIS I																						
+++	2	2					1	1				2	1		1			1	1			
++																						
+	1					1			1													
±												2				2				1	1	
−	3					3					3											
TOTAL . . .	6	2				4	1	1	1		3	4	1		1	2		1	1	1	1	
SYPHILIS II																						
+++	20	8	7	3		2	6	2	8	1	3	5	2	2	1			1	2	1	1	
++	5		1	3		1			3	1	1											
+	1			1					1													
±	2			1	1					2												
−	20				5	15				3	17	1				1						1
TOTAL . . .	48	8	8	8	6	18	6	2	12	7	21	6	2	2	1	1		1	2	1	1	1

TREATED												UNTREATED										
Wassermann	Number	Sachs-Georgi					Meinicke					Number	Sachs-Georgi					Meinicke				
		+++	++	+	±	−	+++	++	+	±	−		+++	++	+	±	−	+++	++	+	±	−

Syphilis III

+++	1	1					1															
++																						
+																						
±																						
−																						
Total . . .	1	1					1															

Latent Syphilis

+++	28	13	8	4	1	2	7	4	10	5	2	2		1	1			1	1			
++	5		2	1	1	1		1	1	1	2											
+	7			5		2			5	1	1											
±	7		2	1	4				1	5	1	1				1					1	
−	87			4	6	77			3	10	74											
Total . . .	134	13	12	15	12	82	7	5	20	22	80	3		1	1	1		1	1	1		

Protocol No. IV (*continued*).

Wassermann	Treated: Number	Sachs-Georgi +++	Sachs-Georgi ++	Sachs-Georgi +	Sachs-Georgi ±	Sachs-Georgi −	Meinicke +++	Meinicke ++	Meinicke +	Meinicke ±	Meinicke −	Untreated: Number	Sachs-Georgi +++	Sachs-Georgi ++	Sachs-Georgi +	Sachs-Georgi ±	Sachs-Georgi −	Meinicke +++	Meinicke ++	Meinicke +	Meinicke ±	Meinicke −
Parasyphilis																						
+++	4		4				1	3														
++																						
+																						
±	3			2		1			1	2												
−	1					1				1		1				1						1
Total . . .	8		4	2		2	1	3	1	3		1				1						1
Doubtful																						
+++	24	11	5	6	1	1	3	7	10	2	2											
++	2		1	1						2												
+	6		2	3	1				2	3	1											
±	2					2					2											
−	101			1	3	97			1	8	92											
Total . . .	135	11	8	10	6	100	3	7	13	15	97											
Without a clinical history																						

NON-SYPHILITIC												TOTAL										
Wassermann	Number	Sachs-Georgi					Meinicke					Number	Sachs-Georgi					Meinicke				
		+++	++	+	±	−	+++	++	+	±	−		+++	++	+	±	−	+++	++	+	±	−
+++												88	38	27	16	2	5	19	18	34	10	7
++												12		4	4	2	2		1	4	4	3
+												15		2	9	1	3			9	4	2
±												15		2	4	5	4			1	8	6
−	7					7					7	223			5	14	204			4	22	197
TOTAL . . .	7					7					7	353	38	35	38	24	218	19	19	52	48	215

If we consider +++, ++ and + as a unique group (positive) :

Results in agreement with B. W. given by	S. G. with ant.	L1	87.54 %
» » » »	D. M.	«	L2	82.44 %
± considered as −	S. G.	»	L1	92.64 %
»	D. M.	»	L2	90.09 %
± » +	S. G.	»	L1	90.66 %
»	D. M.	»	L2	87.54 %
± be neglected	S. G.	»	L1	95.30 %
»	D. M.	»	L2	94.64 %

The same examinations were carried out with the antigen W. 33 and W. 34 — see Protocol No. IV 2.

Antigen S.G. W 33.
Antigen D.M. W 34.

Protocol No. IV 2. 23/VIII-23/IX 1922.

Wassermann	TREATED Number	TREATED Sachs-Georgi +++	++	+	±	—	TREATED Meinicke +++	++	+	±	—	UNTREATED Number	UNTREATED Sachs-Georgi +++	++	+	±	--	UNTREATED Meinicke +++	++	+	±	—
Syphilis I																						
+++	2	2					1	1				2	1		1			1	1			
++																						
+	1					1			1													
±																						
—	3					3					3	2					2				1	1
Total . . .	6	2				4	1	1	1		3	4	1		1		2	1	1		1	1
Syphilis II																						
+++	20	12	4	2	1	1	6	7	5	1	1	5	3	1	1			3	1	1		
++	5		2	2		1			4		1											
+	1			1					1													
±	2			1	1				2													
—	20			3	1	16				3	17	1					1					1
Total. . .	48	12	6	9	3	18	6	7	12	4	19	6	3	1	1		1	3	1	1		1

	TREATED											UNTREATED										
Wassermann	Number	Sachs-Georgi					Meinicke					Number	Sachs-Georgi					Meinicke				
		+++	++	+	±	–	+++	++	+	±	–		+++	++	+	±	–	+++	++	+	±	–
Syphilis III																						
+++	1	1					1															
++																						
+																						
±																						
–																						
Total . . .	1	1					1															
Latent Syphilis																						
+++	28	12	4	7	2	3	10	8	6	2	2	2		2			1	1				
++	5	1		2	1	1		1	2	1	1											
+	7		1	4	1	1			4	3												
±	7		1	2	3	1			3	3	1	1			1			1				
–	87			15	13	69			3	5	79											
Total . . .	134	13	6	30	20	75	10	9	18	14	83	3		2	1		1	2				

Protocol No. IV 2 (*continued*).

Wassermann	TREATED Number	Sachs-Georgi +++	Sachs-Georgi ++	Sachs-Georgi +	Sachs-Georgi ±	Sachs-Georgi −	Meinicke +++	Meinicke ++	Meinicke +	Meinicke ±	Meinicke −	UNTREATED Number	Sachs-Georgi +++	Sachs-Georgi ++	Sachs-Georgi +	Sachs-Georgi ±	Sachs-Georgi −	Meinicke +++	Meinicke ++	Meinicke +	Meinicke ±	Meinicke −
LATENT SYPHILIS (*continued*)																						
+++	4	1		3			1		3													
++																						
+																						
±	3			1	2					2	1											
−	1					1					1	1					1					1
TOTAL . . .	8	1		4	2	1	1		3	2	2	1					1					1
DOUBTFUL												**NO ANAMNESIS**										
+++	24	10	7	5	1	1	10	3	10	1												
++	2		1		1			1		1												
+	6		1	4	1				4	1	1											
±	2				1	1				2												
−	101			2	13	86			1	3	97											
TOTAL . . .	135	10	9	11	17	88	10	4	15	8	98											

NOT SYPHILITIC												TOTAL										
Wassermann	Number	Sachs-Georgi					Meinicke					Number	Sachs-Georgi					Meinicke				
		+++	++	+	±	−	+++	++	+	±	−		+++	++	+	±	−	+++	++	+	±	−
LATENT SYPHILIS (*continued*)																						
+++												88	42	16	21	4	5	33	22	25	5	3
++												12		4	4	2	2		2	6	2	2
+												15		2	9	2	2			10	4	1
±												15		1	4	7	3			5	7	3
−	7					7					7	223			10	27	186			4	12	207
TOTAL . . .	7					7					7	353	42	23	48	42	198	33	24	50	30	216

If we consider +++, ++ and + as a unique group (positive):

Results in agreement with B. W. given by S. G. with ant. W. 33 : 82.44 %
» » » » D. M. with ant. W. 34 : 88.39 %
» » if ± considered as − S. G. » W. 33 : 90.94 %
» » » » D. M. » W. 34 : 92.64 %
» » » » + S. G. » W. 33 : 86.12 %
» » » » D. M. » W. 34 : 92.92 %
» » if ± be neglected : S. G. » W. 33 : 93.73 %
» » » » D. M. » W. 34 : 96.83 %

REPORT ON THE SERODIAGNOSIS OF SYPHILIS

By Dr. L. W. Harrison (London).

In accordance with the wishes of the Medical Research Council, I undertook the general supervision of the research in Great Britain.

A laboratory was fitted up in St. Thomas's Hospital Medical School. Dr. Wyler performed all the tests, and one assistant washed the glass-ware, prepared the apparatus and inactivated the sera.

The specimens were mainly drawn from patients in St. Thomas's Hospital, but a few were posted at my request from other centres where the clinicians' opinions were reliable. A clerk received the specimens in the first instance; changed the identification number by which the clinical history of the case could be traced; gave the serum a new number; entered all particulars in a special book which was kept locked up when not in use; and eventually added the results of the tests as recorded in Dr. Wyler's laboratory book.

Owing to delay in arrival of apparatus and to Dr. Wyler being the only person who conducted the tests, the number of sera so far tested is comparatively small.

The results of the tests are shown in the following tables.

Comparison of Results by the Bordet-Wassermann (W. R.), Sachs-Georgi (S.G.), Meinicke (M.) and Sigma Tests.

Table I.

Sera.

Untreated cases		Number of cases	W. R. Positive	W. R. Doubtful	S. G. Positive	S. G. Doubtful	M. Positive	M. Doubtful	Sigma Positive	Sigma Doubtful
—			—	—	—	—	—	—	—	—
Syphilis I.	with four complete tests.	13	5	2	6	1	7	0	7	0
	with one or more tests omitted (1)	0	0	0	0	0	0	0	0	0
Syphilis II.	with four complete tests.	17	17	0	17	0	17	0	17	0
	with one or more tests omitted	1	1	0	—	—	1	0	1	0
Syphilis III.	with four complete tests.	15	11	1	12	2	12	0	12	0
	with one or more tests omitted	1	1	0	—	—	—	—	1	0

(1) Tests omitted are those in which on account of unsatisfactory control or for other reasons, a report could not be given. They are indicated in the table by a dash (—).

Table I (*continued*).

Untreated cases		Number of cases	W. R. Positive	W. R. Doubtful	S. G. Positive	S. G. Doubtful	M. Positive	M. Doubtful	Sigma Positive	Sigma Doubtful
Syphilis spinal and cerebral (excluding tabes and D. p.)	with four complete tests.	1	1	0	1	0	1	0	1	0
	with one or more tests omitted	0	0	0	0	0	0	0	0	0
Syphilis congenital	with four complete tests.	2	2	0	2	0	2	0	2	0
	with one or more tests omitted	0	0	0	0	0	0	0	0	0
Syphilis Latent	with four complete tests.	22	16	4	21	1	19	1	20	0
	with one or more tests omitted	1	1	0	1	0	—	—	1	0
		1	1	0	—	—	—	—	1	0
Tabes	with four complete tests.	4	3	1	3	0	4	0	4	0
	with one or more tests omitted	1	0	0	1	0	0	0	—	—
Total untreated	with four complete tests.	74	55	8	62	4	62	1	63	0
		1	1	0	—	—	1	0	1	0
	with one or more tests omitted	2	2	0	—	—	—	—	2	0
		1	1	0	1	0	—	—	1	0
		1	0	0	1	0	0	0	—	—
Treated Syphilis (analysed in Table II).	with four complete tests.	403	117	62	202	42	185	28	212	21
	with one or more tests omitted.	7	2	0	—	—	4	1	3	0
		10	5	1	—	—	—	—	6	0
		10	1	0	3	2	1	3	—	—
Syphilis Totals	with four complete tests.	477	172	70	264	46	247	29	275	21
		8	3	0	—	—	5	1	4	0
	with one or more tests omitted	12	7	1	—	—	—	—	8	—
		1	1	0	1	0	—	—	1	0
		11	1	0	4	2	1	3	—	—
Controls (see Notes)	with four complete tests.	226	9	5	18	15	14	9	14	3
		10	0	0	—	—	0	2	0	0
	with one or more tests omitted	7	0	0	—	—	—	—	3	0
		1	0	0	0	1	—	—	0	0
		2	0	0	0	0	0	0	—	—

Table II.

Analysis of treated cases shown in Table I.

Sera.

Treated cases		Number of cases	W. R. Positive	W. R. Doubtful	S. G. Positive	S. G. Doubtful	M. Positive	M. Doubtful	Sigma Positive	Sigma Doubtful
Syphilis I	with four complete tests.	73	5	2	8	8	8	3	7	5
	with one or more tests omitted (1).	2	0	0	—	—	0	0	0	0
		3	0	1	—	—	—	—	1	0
		3	0	0	0	1	0	1	—	—

(1) Tests omitted are those in which, on account of unsatisfactory control or for other reasons, a report could not be given. They are indicated in the table by a dash (—).

Table II (*continued*).

Treated cases		Number of cases	W. R. Positive	W. R. Doubtful	S. G. Positive	S. G. Doubtful	M. Positive	M. Doubtful	Sigma Positive	Sigma Doubtful
		—	—	—	—	—	—	—	—	—
Syphilis II.	with four complete tests.	79	16	7	29	6	24	4	30	3
	with one or more tests omitted	2	1	0	—	—	2	0	1	0
		1	0	0	—	—	—	—	0	0
		3	0	0	1	0	0	2	—	—
Syphilis III.	with four complete tests.	96	45	17	70	7	65	6	71	3
	with one or more tests omitted	1	0	0	—	—	0	1	0	0
		2	2	0	—	—	—	—	2	0
		1	0	0	1	0	0	0	—	—
Syphilis spinal and cerebral (excluding tabes and G. P. I.)	with four complete tests.	12	6	3	7	2	6	1	8	1
	with one or more tests omitted	1	1	0	—	—	—	—	1	0
Syphilis congenital.	with four complete tests.	17	10	5	14	2	16	0	16	0
	with one or more tests omitted	1	0	0	—	—	1	0	1	0
		1	1	0	—	—	—	—	1	0
Syphilis Latent	with four complete tests.	102	21	22	54	14	46	13	61	6
	with one or more tests omitted	2	0	0	0	1	0	0	—	—
		2	1	0	—	—	—	—	1	0
Tabes	with four complets tests.	21	12	5	17	3	17	1	16	3
	with one or more tests omitted	1	1	0	—	—	1	0	1	0
		1	1	0	1	0	1	0	—	—
G. P. I.	with four complete tests.	3	2	1	3	0	3	0	3	0
Total treated.	with four complete tests.	403	117	62	202	42	185	28	212	21
	with one or more tests omitted	7	2	0	—	—	4	1	3	0
		10	5	1	—	—	—	—	6	0
		10	1	0	3	2	1	3	—	—

Table III.

Spinal fluids.

Treated cases		Number of cases	W. R. Positive	W. R. Doubtful	S. G. Positive	S. G. Doubtful	M. Positive	M. Doubtful	Sigma Positive	Sigma Doubtful
		—	—	—	—	—	—	—	—	—
Syphilis I.	with four complete tests. with one or more tests omitted (1)									
Syphilis II.	with four complete tests. with one or more tests omitted					Nil.				
Syphilis III.	with four complete tests. with one or more tests omitted									

(1) Tests omitted are those in which, on account of unsatisfactory control or for other reasons, a report could not be given. They are indicated in the table by a dash (—).

Table III (*continued*).

Treated cases		Number of cases	W. R. Positive	W. R. Doubtful	S. G. Positive	S. G. Doubtful	M Positive	M Doubtful	Sigma Positive	Sigma Doubtful
Syphilis spinal and cerebral (excluding tabes and D. p.)	with four complete tests.									
	with one or more tests omitted									
Syphilis congenital	with four complete tests.					Nil.				
	with one or more tests omitted									
Syphilis Latent	with four complete tests.									
	with one or more tests omitted									
Tabes	with four complete tests.	1	1	0	1	0	1	0	1	0
G. P. I.	with four complete tests.	3	3	0	2	1	2	1	2	0
Total treated	with four complete tests.	4	4	0	3	1	3	1	3	0

Notes I. — In the above tables the following were classed as *doubtful.*

W. R.=Distinct but incomplete lysis with standard amount of complement. In a treated case this is interpreted in the same light as Sigma, units 1.1 to 1.5 at 40 hours. In an untreated case, the test would be repeated, being regarded as highly suspicious.

S. G ± or + flocculation

M. ± or + flocculation.

Sigma : 1.1 to 1.5 units at 40 hours. See note on W. R. re interpretation in treated and untreated cases.

II. *Controls.* — The cases entered under the heading of controls were those in which the examination revealed no history or outward evidence of syphilis. In eleven cases the unanimity of the tests in giving positive reactions indicated that, in spite of the negative history and clinical evidence, the patient was really suffering from syphilis.

The following notes refer only to cases which gave a definite positive with one or more tests.

No. 95. reported on account of Gonorrhœa on 12/6/22. His blood serum as No. 95 on 17/6/22 gave :

W. R. negative.

S. G.+++ (24 hours).

M. ± (»).

Sigma 1.5 (20 hours).

He was re-examined on 18/7/22 and still showed no evidence of syphilis. The blood serum tested as No. 385 gave :

W. R. negative.

S. G. + (24 hours).

M. + (»).

Sigma 1.3 (21 hours).

Nos. 222 and 327. — Patient, aged 29, who had a sore in 1918 diagnosed as chancroid and treated by local applications only. His W. R. was subsequently negative on five occasions. He reported on 28/6/22, for "pain in testicle" and showed no signs of syphilis. He was tested as No. 222 in this series and gave :

W. R. negative.
S. G. ± but serum control flocculated.
M. negative.
Sigma 0 units (20 hours).

He received an injection of 0.45 gm. 914 as a provocative on 5/7/22 and his serum, tested as No. 327 on 12/7/22, gave :

W. R. negative.
S. G. control failed.
M. negative.
Sigma 0 units (21 hours).

No. 373. — Reported on 17/7/22 with discharge from prepuce of one month's duration. The prepuce could not be retracted, but no sore was felt and there were no other signs of syphilis. The patient was transferred to another centre and has not yet been traced. The reactions were :

W. R. negative.
S. G. + = doubtful.
M. negative.
Sigma 6.8 (21 hours).

No. 394. — Reported for examination having had gonorrhœa six months previously. No history or signs of syphilis.

Blood serum gave :
W. R. negative.
S. G. + = doubtful.
M. + = »
Sigma 1.6 (21 hours).

No. 483 and 799. — Reported for a pain in the groin. No history or signs of syphilis; had been examined a year previously with same result.

Blood serum, as No. 483, gave :

W. R. negative.
S. G. control failed.
M. control failed.
Sigma 2.5 (21 hours).

As No. 799 two months later, during which no treatment, the serum gave :

W. R. negative.
S. G. »
M. »
Sigma 0.0 at 44 hours.

No. 485 and 733. — Reported on account of acute gonorrhœa No history or signs of syphilis.
Blood serum as No. 485 gave :

W. R. negative.
S. G.+++ but serum control showed marked flocculation.
M.++ but serum control showed some flocculation.
Sigma 2.5 (21 hours).

The same patient's serum tested one month later as No. 733 gave :

W. R. negative.
S. G. »
M. »
Sigma 0.0 units (20 and 44 hours).

No. 574. — Case of gonococcal arthritis. No history or signs of syphilis. Blood serum gave :

W. R. negative.
S. G.++ but control tube flocculated.
M.++ but control tube flocculated.
Sigma 2.5 (20 hours).

Patient to be seen again on October 16th and serum will be retested.

No. 614. — Gonorrhœa (Prostatic abscess). No history or sign of syphilis.
Blood serum gave :

W. R. negative.
S. G.++.
M. negative.
Sigma 0 (20 hours).
» 0.62 (44 hours).

No. 741. — Gonorrhœa. No history or signs of syphilis.
Blood serum gave :

W. R. negative.
S. G. »
M.++
Sigma 0 (20 and 44 hours).

No. 847. — Fatal case of retention of urine. No history or signs of syphilis. Post-mortem examination revealed atheroma of aorta but no evidence of syphilis.

W. R. negative.
S. G. no test.
M.++++ with 1.5 cc. extract only.
Sigma 0. (21 hours).
» 0.78 (44 hours).

No. 989. — Enlarged prostate. No history or signs of syphilis. Blood serum gave :

W. R. negative.
S. G. »
M.++++ with 1.5 c.c. extract only.
Sigma 0. (20 hours).
» 0.78 (40 hours).

In the following cases the unanimity of the results indicates that the patient really was suffering from syphilis. Note the effect of treatment in case tested as Nos. 206, 250 and 589.

No. 10. — Attended for prophylactic treatment. Gave a history of having suffered from gonorrhœa. No clinical evidence of syphilis. Results of tests :

W. R.++
S. G.++++
M.+++
Sigma 24.1 (7 hours).
» 41.4 (24 hours).

No. 464. — Gonorrhœa. No clinical evidence of syphilis. Patient cannot be traced.
Results of tests :

W. R.++
S. G.++++
M.+++
Sigma 3.2 (22 hours).

No. 501. — "Carcinoma of Prostate". No history of sore, but of gonorrhœa.
Results of tests :

W. R.++
S. G.++++
M.++++
Sigma 63 (21 hours).

Nos. 618 and 675. — Gonorrhœa. No clinical evidence of syphilis acquired or congenital, which was denied strongly.
Results of tests :

As No. 618	As No. 675 (one week later).
W. R. +±	+±
S. G. ++++	++++
M. ++++	++++
Sigma 18.1 (20 hours).	16.6 (20 hours).

No. 736. — Gonorrhœa. Also 11 years' history of gonorrhœa. No sign of syphilis.
Results :

W. R.±
S. G.+++
M.++
Sigma 3.0 (20 hours).

No. 685. — "Albuminuria". No history of syphilis, but gonorrhœa 18 years previously.

Results of tests :

W. R.±
S. G.++++
M.++++
Sigma 13.7 (20 hours).

Nos. 206, 250 and 589. — "Chronic gonorrhœa". No signs or history of syphilis, which was denied strongly.

Results of test :

As 206	As 250
W. R.++	++
S. G.+++	+++
M.+++	++++
Sigma 12.4 (20 hours)	12.4 (21 hours).

Case was put on treatment and after 1.9 gm. "914" with Hg. 0.3 gm. as No. 589, results were :

W. R.++
S. G.+++
M.+++
Sigma 5.4 (20 hours).

No. 221. — Reported with gonorrhœa. No clinical evidence of syphilis. Patient confident he never had any sore or rash.

Results of tests :

W. R.++
S. G.++++
M.++++
Sigma 119.8 (20 hours).

The following non-syphilitic cases gave positive flocculation in the S. G. test, but the control tube also showed flocculation to the degree shown under the columns headed S. C. The results by the other tests are shown for comparison.

T = test : S. C. = serum control.

Test No.	W. R.		S. G.		M.		Sigma	
	T.	S. C.	T.	S. C.	T.	S. C.		
No. 141	—	—	+++	—	—	±	0.66	(20 hours)
406	—	—	++	±	—	—	0.	(21 hours)
420	—	—	++	—	—	±	0.	(21 hours)
433	—	—	++	—	—	—	0.	(21 hours)
482	—	—	++	±	—	—	0.78	(44 hours)
484	—	—	+++	—	—	—	1.3 0.62	(21 hours) (44 hours)
523	—	—	++	++	—	±	0.75 0.62	(20 hours) (43 hours)
568	—	—	++	+	—	±	1.3 0.0	(20 hours) (44 hours)
871	—	—	++	±	—	—	0. 0.62	(21 hours) (44 hours)

No. 482 was retested as No. 722 and gave the following :

W. R. negative.
S. G. »
M. +
Sigma 0.0 (20 hours).
» 0.78 (44 hours).

From the above it seems probable that the following were non-specific reactions :

Sigma. . . . Nos. 394, 483 (see 799 from same patient), 485 (see 733 from same patient), and 574. Possibly No. 373 was a case of primary syphilis.
S-G. . . . . Nos. 95 and 614.
M. . . . . Nos. 741, 847 (?) and 989 (?).

It seems worthy of note that in Nos. 483, 485 and 574 where the Sigma test gave a positive reaction the serum control failed in the S.-G. and M. tests.

Agreement of results with Syphilitic sera.

In the Appendix is given a number of tables showing how results agreed with one another.
Generally they show :

Agreement of W. R. and Sigma tests in

89.1 per cent. untreated cases.
80.6 » treated cases.

Agreement of W. R. and S.-G. tests in

87.8 per cent. untreated cases.
80.6 » treated cases.

Agreement of W. R. and M. tests in

87.8 per cent. untreated cases.
87.8 » treated cases.

Compared in respect of delicacy, the percentages of positives to each test were :

	W. R.	Sigma	M.	S. G.
Untreated	74.3	85.1	83.7	83.7
Treated	43.6	58.5	52.6	60.7

showing generally a larger percentage of positives by the flocculation tests. In making up this table, doubtful reactions were counted as negative in untreated cases and as positive in treated cases. Against this greater delicacy of the flocculation tests must be set the non-specific results mentioned above.

In a certain number of cases where the W. R. was negative and even also the flocculation tests, the W. R. had been tested in another laboratory by the cold fixation method and found to be positive.

Repeated tests on the same sera.

In a number of cases the same patient's serum was submitted to the four tests at different periods and sometimes the results were very interesting, as shown below, where W. R.++ S. G.++++ and M.++++ indicate maximum strengths of reaction.

Primary cases.

	W. R.	S. G.	M.	Sigma units	
No. 80 as an untreated case gave	++	++++	++++	208	
After receiving 2.55 gm. "914" and Hg. injections for two months he gave, as *No. 510.*	—	—	—	0.62	
No. 109 a treated case gave	±	—	—	0	(20 hours)
After seven weeks rest from treatment he gave as *No. 382* . .	±	+	++	1.7	(20 hours)
No. 432 a treated case gave	—	(control failed)	+	0.75	(21 hours)
After six weeks rest from treatment he gave, as *No. 780* . .	—	—	—	0.62	

Five other primary cases were each tested twice but gave the same negative results.

Secondary cases.

	W. R.	S. G.	M.	Sigma units
No. 22 a treated case, gave . .	++	++++	++++	318
After receiving 2.8 gm. "914" and Hg. injections in 3 months he gave, as *No.* 684	—	++	++	7.4

One other case tested twice gave exactly the same positive result. Three tested three times and one tested twice gave practically the same negative results.

Tertiary cases.

	W. R.	S. G.	M.	Sigma units	
No. 12 a treated case gave	—	++	±	0.75	(20 hours)
After taking pills for about a month he gave as *No. 260.* . .	+	++	—	1.5	(20 hours)
	—	(control failed)			

	W. R.	S. G.	M.	Sigma units
After a further course of pills for about a month he gave, as *No. 640* .	−	−	−	2.0
No. 27 a treated case gave	++	++++	++++	58.0
A week later, as *No. 50*. .	++	++++	++++	58.0
After receiving 2.8 gm. silver - salvarsan, he gave as *No. 644*. . .	++	++++	++++	44.5
No. 603 an untreated case	±	+++	++++	7.4
Four days after 0.45 gm. "914" as *No. 648* (provocative effect) . . .	++	+++	+++	13.7
No. 274 a treated case gave	±	++	+++	3.6
After eight weeks on no treatment, as *No. 751*.	±	++	++	2.0
No. 415 a treated case gave	±	++	++++	16.6
After five weeks taking pills as *N° 711* . . .	±	++++	++++	8.3
No. 304 a treated case gave	−	++	−	1.7
After two months on no treatment, as *No. 820*.	−	−	−	0.94
No. 507 a treated case gave	++	+++	+++	6.8
After eight weeks on no treatment, as *No. 962*.	+±	− (1)	+++	8.3
No. 242 a treated case gave	++	+++	++++	7.4
After two months on silver - salvarsan, as *No. 990*.	++	+++	++++	24.0

One case tested three times at intervals of three months and two weeks respectively on no treatment gave the same negative results.

One case tested twice at an interval of three months on no treatment gave the same negative results.

Congenital cases.

	W. R.	S. G.	M.	Sigma units
No. 400 treated up to six months previously . .	±	+	++++	16.6
After six months on no treatment, as *No. 800*.	±	+++	++++	6.8

Latent cases.

No. 52 a treated case gave	−	−	−	0	(20 hours)
After six weeks on medicine as *No. 344*. . .	−	±	—	0	(20 hours)

(1) All S. G. tests in batch including 962 were apparently unduly weak.

	W. R.	S. G.	M.	Sigma units	
No. 70 a treated case gave	±	−	−	0	(20 hours)
After one month, having received 1.3 gm. "914" he gave as *No. 337* . .	−	±	−	0	(20 hours)
No. 108 a treated case gave	++	−	+	1.6	
After receiving 1.35 gm. "914" he gave as *No. 319*.	±	++	−	1.6	
No. 206 an untreated case gave	++	+++	+++	7.4	
As *No. 250* still an untreated case he gave. .	++	+++	++++	7.4	
After having received 1.9 gm. "914" he gave as *No. 589*.	++	+++	+++	5.4	
No. 256 a treated case gave	−	−	−	0	(20 hours)
After seven weeks on no treatment, as *No.627*.	−	−	−	1.3	
No. 284 a treated case gave	±	+	+	1.7	
After six weeks on pills as *No. 672*.	−	−	+	1.4	(20 hours)
No. 331 an untreated case gave	±	+++	++++	6.7	
After eight weeks on no treatment, as *No.688*.	±	++	+++	3.6	
No. 389 a treated case gave	−	−	−	0	(20 hours)
After one month on no treatment, as *No.593*.	−	++	+	2.3	
No. 398 a treated case gave	−	+++	+++	1.9	
After receiving 1.95 gm. "914" as *No. 660*. . .	±	+	++	2.3	
After receiving a further 0.6 gm. "914" in one week, as *No. 735*. . .	±	++	+	1.7	
No. 426 a treated case gave	−	−	+	0	(20 hours)
After three weeks on pills as *No. 604*.	−	−	−	0.62	
No. 456 a treated case gave	−	+++ (control bad)	± control bad)	3.2	
After nine months on no treatment as *No. 661*.	±	+++	+++	3.6	
No. 656 an untreated case gave	++	++++	+++	6.8	
After two weeks on no treatment, as *No.790*.	±	++	+++	1.9	
No. 56 a treated case gave	−	+++	−	2.1	
After two months on pills, as *No. 784*.	−	++	++	3.2	
No. 391 a treated case gave	±	±	+++	3.2	
After six weeks having received 0.9 gm. "914" he gave as *No. 743* . .	±	+++	+++	2.5	

	W. R.	S. G.	M.	Sigma units
	—	—		—
No. 474 a treated case gave	—	+	—	1.1
After five weeks on no treatment as *No. 757*.	—	—	—	0.94
No. 429 a treated case gave	—	+++	++ +	1.7
After receiving 3.15 gm. "914" he gave as *No. 809*	—	++	++	1.9
No. 255 a treated case gave	—	—	+	3.6
After two months on pills as *No. 966*.	—	++	++	3.9
No. 98 (1) an untreated case gave.	++	++++	++++	45.5
As *No. 152* still an untreated case, he gave .	++	++++	++++	31.0
As *No. 217* still an untreated case, he gave.	++	++++ (control bad)	++++ (control bad)	39.0

Dr. Wyler's remarks on page 299 include an estimate of the time involved in the conduct of the various tests. This is of practical importance since the greater the time absorbed in serum tests for syphilis the greater the charge which will be made by serologists to public health authorities who provide for the examination of sera at the public expense. If, for example, the British Health Authority were asked to substitute one of the flocculation tests for the Wassermann and the former cost more, it would be necessary first to decide whether or not the new test was so much superior to the Wassermann as to be worth the additional cost.

The importance of this will be realised when it is added that the public health authorities in England paid for serum tests in 1921 approximately the sum of £ 36,000.

Nov. 13/1922.

(Sd.) L. W. HARRISON.

APPENDIX.

Results of Tests on Sera from 74 Untreated cases and *403 Treated* cases of Syphilis.

All four tests agreed.

	Prim.	Sec.	Tert.	Spin. & Cere. (excldg. Tabes & D. p.)	Congen.	Latent	Tabes	D. p.	Total syph.
	—	—	—	—	—	—	—	—	—
Untreated pos. . .	5	17	10	1	2	15	3	0	53
— neg. . .	6	0	2	0	0	1	0	0	9
Treated pos. . . .	5	21	59	6	15	37	15	3	161
— neg. . . .	54	41	16	2	1	23	0	0	137

(1) This case denies ever having syphilis, but his wife has a ++ W. R.

That is total agreement of all four tests in 83.7 per cent. of untreated cases and in 74.1 per cent. of treated cases.

Sigma, S. G. and M. positive with W. R. negative.

	Prim.	Sec.	Tert.	Spin. & Cere. (excldg. Tabes & G. P. I.)	Congen.	Latent	Tabes	D. p.	Total syph.
Untreated	1	0	1	0	0	4	0	0	6
Treated	1	4	8	0	1	17	2	0	33

That is total agreement of Sigma, S. G. and M. tests in 91.8 per cent. of untreated cases and in 82.3 per cent. of treated cases.

Sigma and S. G. positive with M. and W. R. negative.

	Prim.	Sec.	Tert.	Spin. & Cere. (excldg. Tabes & G. P. I.)	Congen.	Latent	Tabes	D. p.	Total syph.
Untreated	0	0	0	0	0	0	0	0	0
Treated	7	4	4	1	0	5	0	0	21

That is agreement of Sigma and S. G. tests in 91.8 per cent. of untreated cases and in 87.3 per cent. of treated cases.

Sigma positive with S. G., M. and W. R. negative.

	Prim.	Sec.	Tert.	Spin. & Cere. (excldg. Tabes & G. P. I.)	Congen.	Latent	Tabes	G. P. I.	Total syph.
Untreated	0	0	0	0	0	0	0	0	0
Treated	1	2	1	0	0	6	1	0	11

That is Sigma positive in a further 0.0 per cent. of untreated cases and in a further 2.7 per cent. of treated cases.

Sigma and M. positive with S. G. and W. R. negative.

	Prim.	Sec.	Tert.	Spin. & Cere. (excldg. Tabes & G. P. I.)	Congen.	Latent	Tabes	G. P. I.	Total syph.
Untreated	1	0	0	0	0	0	1	0	2
Treated	1	0	2	0	0	1	0	0	4

That is Sigma and Meinicke positive in a further 2.7 per cent. of untreated cases and in a further 0.9 per cent. of treated cases.

Sigma, M., and W. R. positive with S. G. negative.

	Prim.	Sec.	Tert.	Spin. and Cere.	Congen.	Latent	Tabes	G. P. I.	Total syph.
Untreated	0	0	1	0	0	0	0	0	1
Treated	0	1	0	1	0	0	0	0	2

Sigma, S. G. and W. R. positive with M. negative.

	Prim.	Sec.	Tert.	Spin. and Cere.	Congen.	Latent	Tabes	G. P. I.	Total syph.
Untreated	0	0	0	0	0	1	0	0	1
Treated	0	1	0	1	0	0	0	0	2

W. R., S. G., M. positive with Sigma negative.

	Prim.	Sec.	Tert.	Spin. and Cere.	Congen.	Latent	Tabes	G. P. I.	Total syph.
Untreated	0	0	0	0	0	0	0	0	0
Treated	2	0	1	0	0	1	1	0	5

S. G. and M. positive with W. R. and Sigma negative.

	Prim.	Sec.	Tert.	Spin. & Cere.	Congen.	Latent	Tabes	G. P. I.	Total syph.
Untreated	0	0	0	0	0	0	0	0	0
Treated	1	2	1	0	0	0	0	0	4

S. G. positive with M., W. R. and Sigma negative.

	Prim.	Sec.	Tert.	Spin. & Cere.	Congen.	Latent	Tabes	G. P. I.	Total syph.
Untreated	0	0	1	0	0	1	0	0	2
Treated	1	3	4	0	0	7	1	0	16

M. positive with S. G., W. R. and Sigma negative.

	Prim.	Sec.	Tert.	Spin. & Cere.	Congen.	Latent	Tabes	G. P. I.	Total syph.
Untreated	0	0	0	0	0	0	0	0	0
Treated	0	0	0	0	0	1	0	0	1

W. R., S. G. positive with M. and Sigma negative.

	Prim.	Sec.	Tert.	Spin. & Cere.	Congen.	Latent	Tabes	G. P. I.	Total syph.
Untreated	0	0	0	0	0	0	0	0	0
Treated	0	0	0	1	0	1	0	0	2

W. R. positive with S. G., M. and Sigma negative.

	Prim.	Sec.	Tert.	Spin. & Cere.	Congen.	Latent	Tabes	G. P. I.	Total syph.
Untreated	0	0	0	0	0	0	0	0	0
Treated	0	0	0	0	0	2	0	0	2

W. R., M. and Sigma positive with S. G. negative.

	Prim.	Sec.	Tert.	Spin. & Cere.	Congen.	Latent	Tabes	G. P. I.	Total syph.
Untreated	0	0	0	0	0	0	0	0	0
Treated	0	0	0	0	0	1	0	0	1

W. R., S. G. and Sigma positive with M. negative.

	Prim.	Sec.	Tert.	Spin. & Cere.	Congen.	Latent	Tabes	G. P. I.	Total syph.
Untreated	0	0	0	0	0	0	0	0	0
Treated	0	0	0	0	0	0	1	0	1

REPORT ON SACHS-GEORGI, MEINICKE AND DREYER-WARD FLOCCULATION REACTIONS

Carried out, under the direction of L. Hirszfeld,
by Z. Milinska *and* Z. Modrzewska

State Epidemiological Institute, Warsaw.

General Remarks

The choice of material used for the experiments was made in accordance with the rules laid down in London. The doctors were requested to fill in carefully the forms which accompanied the sera. When the clinical table was not sufficiently clear or when there was no history the case was registered as "doubtful" or "without history". The results were read by an assistant, who did not know the result of the Wassermann reaction or the text of the bulletin which accompanied the serum. Finally, I personally verified the series of reactions.

For reasons over which we had no control, the reactions were first made with our own antigen. Meinicke original antigen was at our disposal only on March 28th and Sachs-Georgi on April 13th. Reactions were carried out with these two antigens for a fairly long period. In order to give an idea of the results as a whole, we have given in Table I those produced by our antigen, and a special table is given showing the comparative value of our own antigen and the Sachs-Georgi and Meinicke antigens. We could not undertake parallel experiments with the Dreyer-Ward method as the necessary apparatus was placed at our disposal only in May. It was not until August that we were able to carry out tests according to the Σ method, and our sera were examined both by the Dreyer-Ward and the Sachs-Georgi methods (original antigens). This last series includes 500 cases. The results of the tests made according to the Sachs-Georgi method are the same in the last series as the results previously obtained; we have therefore not repeated the report.

We will first study the Sachs-Georgi and Meinicke reactions and then take the Σ reaction.

Technique

In the case of the Sachs-Georgi reaction, the antigen was prepared in accordance with Sachs' last prescription, that is to say, one part physiological serum was added to one part alcoholic extract, and four other parts physiological serum were added, these being dropped rapidly into the former. Previous experiments

had confirmed the fact, already stressed by Sachs-Georgi, that the rapidity with which the dilution is made influences the sensitiveness of the antigen. During our experimentation the use of hypertonic salt solutions was of no importance in the specificity of the reactions. Flocculation is sometimes stronger when a salt solution of 1.5% is used. On the other hand, the quantity of serum used greatly influences the reaction. We often found positive sera of which a dose of 0.1 reacted without any clearness, whereas a dose of 0.2 produced strong precipitation. For this reason we used 0.2 serum for all our tests, in accordance with the most recent data by Sachs.

The Meinicke reaction was made in accordance with the rules laid down for the third modification. The antigens were prepared without cholesterine. Canadian "beaume", which was recommended recently, was not utilised. The results were read with a lens and it was noticed that it is a good thing to shade the light slightly with the end of the finger.

Besides the material with fairly clearly established history and clinical tables, we also give the experimentation on the usual material of the institute, which includes doubtful cases or cases without history. We should not like to neglect this material, as it is our task to establish the parallels between fixation reactions and flocculation reactions, and this is very well shown by the use of doubtful material. We do not think, however, that it is necessary to reconsider the forms which accompany the sera, as they contain, for the greater part, suspected history of syphilitic infection, and it is not from these forms that any conclusions could be drawn as to which of the methods of deviation or of flocculation is the most correct. Moreover, we shall show later on that the percentage of cases not concordant with the different stages of syphilis and that of doubtful cases are more or less identical, so that this rule may be regarded as conclusive.

Our control sera came either from hospitals and were guaranteed not to be syphilitic, or they were normal sera from wet-nurses.

When these control sera gave positive reactions with the Bordet-Wassermann method they were considered doubtful.

In these cases of "healty" sera we never found that a negative Wassermann reaction became positive with the Sachs-Georgi or Meinicke methods. The total of the cases studied according to the Meinicke methods is presented in accordance with the outline proposed to us. The tests according to the Sachs-Georgi method were started earlier and lasted longer. This is why we have a larger number of cases for the Sachs-Georgi reaction. When the Meinicke antigen was ready, tests were made according to the two methods, so that the number of cases examined by the Meinicke method corresponds at the same time to the number of cases examined by the two methods (see Table I).

Table I.

Table showing the number of cases examined by the Sachs-Georgi and Meinicke Tests.

	Sachs-Georgi	Meinicke
Syphilis I. untreated	45	22
Syphilis II. untreated	127	89
Syphilis III. untreated	36	25
Syphilis latent untreated	166	102
Parasyphilis untreated	8	8
Syphilis under treatment	1,949	1,229
Total number of cases of syphilis	2,331	1,475
Control	670	481
Total	3,001	1,956
Doubtful cases	1,793	1,186
Total of all cases	4,794	3,142

The exact results of the cases as a whole are given in two special tables. The first table contains the results of the experiments made between January 27th and June 27th, representing the choice of material supervised by myself. The second table contains the experiments made during the summer months by Madame Modrzewska. The two assistants also worked, using the same methods and the same antigens, so that the differences that may possibly appear in the results arise from secondary factors (summer months) and the individualities of the workers.

We shall elaborate in a more detailed manner the material of the first table, as it is this material which was studied and treated most carefully and as it includes the number of experiments called for by the London Conference.

In analysing the results, we have tried to find the comparative value of the reactions of the syphilitic sera from the quantitative point of view. Consequently, we did not restrict ourselves to establishing whether the reaction was positive, but we also noted the degrees of intensity of the complement fixation test and the corresponding degrees of flocculation. We made a distinction between four different degrees of positive reactions (+++, ++, + and ±), according to whether all the antigens or one antigen only reacted, and whether the fixation was complete or followed by a slight hemolysis, or according to the strength of the flocculation.

The annexed tables contain rubrics according to the various stages of syphilis. These tables were drawn up after each series of experiments, so that we were afterwards able to establish easily general tables concerning the varied intensity of the positive reactions by the complement fixation method or flocculation reactions (see Table II).

Table II.

WASSERMANN	NUMBER	TREATED										NUMBER	UNTREATED									
		Sachs-Georgi					Meinicke						Sachs-Georgi					Meinicke				
		+++	++	+	±	−	+++	++	+	±	−		+++	++	+	±	−	+++	++	+	±	−
Syphilis I																						
+++	22	15	3	3		1	13	2	4	1		19	17		2			16	1	1		
++	2		1		1						1											
+	3			2		1				1	1	2		1	1			1			1	
±											1											
−	12		1			11					1	2					2					2
TOTAL. . . .	39	39					25					23	23					22				
Syphilis II																						
		+++	++	+	±	−	+++	++	+	±	−		+++	++	+	±	−	+++	++	+	±	−
+++	166	141	12	7	5	1	122	8	9	3		96	87	7		1	1	76	1	4	2	
++	9	1	3	2	2	1	1		4	2		2		1		1			1			
+	11			9	1	1		1	5	3		1					1			1		
±	13			4	8	1		1		1	2											
−	52				3	49					32	5					5					4
TOTAL. . . .	251	251					194					104	104					89				

WASSERMANN	NUMBER	TREATED										NUMBER	UNTREATED										
		Sachs-Georgi					Meinicke						Sachs-Georgi					Meinicke					
		+++	++	+	±	−	+++	++	+	±	−		+++	++	+	±	−	+++	++	+	±	−	
Syphilis III																							
+++	78	61	10	5	2		56	5	5	3	1	24	16	5	3			19	2	2			
++	8		5	1	1	1	1	3		1	1												
+	5			1	4				1	2													
±	13		1	3	7	2			4	2	2	1					1						1
−	64		1	2	2	59					54	1		1									1
TOTAL. . . .	168	168					142					26	26					25					
Latent Syphilis																							
+++	346	235	51	29	27	4	251	38	37	31	4	95	70	14	5	3	3	64	13	6	7	3	
++	39	3	9	10	10	7		2	7	16	11	6		2	4				2	1	2		
+	46		4	22	15	5		1	17	18	7	2	1			1			1				1
±	46	2	4	10	25	5	3	2	6	11	18	2		1			1		1				1
−	389		2	4	8	375		2	1		368												
TOTAL. . . .	866	866					831					105	105					102					

Parasyphilis

Wassermann	Number	Treated										Number	Untreated									
		Sachs-Georgi					Meinicke						Sachs-Georgi					Meinicke				
		+++	++	+	±	−	+++	++	+	±	−		+++	++	+	±	−	+++	++	+	±	−
+++	25	15	5	3	2		15	1	4	5		6	5	1				6				
++																						
+	3			2		1			1	1	1											
±																						
−	9	1				8		1			8	2					2					2
Total. . . .		37					37						8					8				

		Dourtful											Without clinical history									
		+++	++	+	±		+++	++	+	±			+++	++	+	±	−	+++	++	+	±	−
+++	194	122	24	27	18	3	116	23	20	10	5	229	142	55	20	10	2	118	34	22	8	3
++	23		8	9	6		3	2	6	4	2	34	2	9	14	5	4		5	8	7	3
+	26	1		11	12	2	2	1	3	7	8	38	4	2	16	12	4	1		17	8	3
±	35		1	4	17	13			2	9	20	29		1	3	17	8				5	5
−	424	1	5	4	7	407		1	1	2	393	422	1	3	2	15	400	1		1		297
Total. . . .	702	702					640					752	752					546				

WASSERMANN	NUMBER	NON-SYPHILITICS										NUMBER	TOTAL									
		Sachs-Georgi					Meinicke						Sachs-Georgi					Meinicke				
		+++	++	+	±	-	+++	++	+	±	-		+++	++	+	±	-	+++	++	+	±	-
PARASYPHILIS (*continued*)																						
+++												1,300	926	188	103	67	16	853	128	114	70	16
++												123	6	38	41	25	13	5	16	24	35	16
+												137	6	7	63	46	15	4	3	43	47	22
±	1					1					1	139	2	8	24	73	32	3	4	12	34	48
−	480					480					480	1,864	4	12	14	35	1,799	4	4	3	3	1,652
TOTAL. . . .		481					481					3,563	3,563					3,163				

Total of cases examined by the Sachs-Georgi method 3,563

Results do not agree in . . . . . 141 = 3.9 %

W + S.G − 76 = 2.1 %
W − S.G + 65 = 1.8

Total of cases examined by Meinicke 3,163

Results do not agree in . . . . . 116 = 3.6 %

W + M − 102 = 3.1 %
W − M + 14 = 0.5

Without consideration of ± according to S.G. . . . . 74 = 2 %
Without consideration of ± according to M. . . . . 65 = 2 %

The table contains the first series of experiments as a whole. The number of non-concordant cases with the Sachs-Georgi reaction is 3.9%, of which B-W positive, S-G negative; 76 cases—that is to say 2.1% and B-W negative, S-G positive : 67 cases—that is 1.8%. If the slightly positive cases are not taken into account (74 cases), the figure of 2% non-concordant cases is reached. For the Meinicke reaction, on a total number of 3,163 cases, 116 non-concordant cases were found, that is to say 3.6%, of which B-W positive, M negative; 102 cases, that is 3.1%, B-W negative and M positive—14 cases, that is 0.5%.

If the cases giving slightly positive reactions are not taken into consideration, only 2% (65) non-concordant cases are found (see Table III).

Table

Table III.

Wassermann	Number	TREATED										UNTREATED										
		Sachs-Georgi					Meinicke					Number	Sachs-Georgi					Meinicke				
		+++	++	+	±	−	+++	++	+	±	−		+++	++	+	±	−	+++	++	+	±	−
Syphilis I																						
+++	9	8			1							15	14	1								
++	3			3								1		1								
+	3	1			2							2				2						
±	1		1									1				1						
−	5				1	4						3					3					
Total . . .	21											22										
Syphilis II																						
+++	17	14	1	2								21	15	5	1							
++	1			1								1		1								
+	1					1																
±	3	1	1	1																		
−	1					1						1					1					
Total . . .	23											23										

Wassermann	TREATED Number	Sachs-Georgi +++	Sachs-Georgi ++	Sachs-Georgi +	Sachs-Georgi ±	Sachs-Georgi −	Meinicke +++	Meinicke ++	Meinicke +	Meinicke ±	Meinicke −	UNTREATED Number	Sachs-Georgi +++	Sachs-Georgi ++	Sachs-Georgi +	Sachs-Georgi ±	Sachs-Georgi −	Meinicke +++	Meinicke ++	Meinicke +	Meinicke ±	Meinicke −
SYPHILIS III																						
+++	3	3										9	6	2			1					
++																						
+	3			1	2																	
±	1					1																
−	3				1	2						1					1					
TOTAL . . .	10											10										
LATENT SYPHILIS																						
+++	132	101	19	10	2							55	53	2								
++	33	13	10	7	2	1						2		2								
+	36	2	4	10	11	9						3	2		1							
±	34	4	1	8	14	7						1			1							
−	285	3	4	10	19	262																
TOTAL . . .	533											61										

Wassermann	TREATED Number	Sachs-Georgi +++	Sachs-Georgi ++	Sachs-Georgi +	Sachs-Georgi ±	Sachs-Georgi −	Meinicke +++	Meinicke ++	Meinicke +	Meinicke ±	Meinicke −	UNTREATED Number	Sachs-Georgi +++	Sachs-Georgi ++	Sachs-Georgi +	Sachs-Georgi ±	Sachs-Georgi −	Meinicke +++	Meinicke ++	Meinicke +	Meinicke ±	Meinicke −
PARASYPHILIS																						
+++																						
++																						
+																						
±	1				1																	
−																						
TOTAL . . .																						

	DOUBTFUL	+++	++	+	±	−	+++	++	+	±		WITHOUT CLINICAL HISTORY	+++	++	+	±	−	+++	++	+	±	−
+++	8	1		4	1	2						66	47	7	8	3	1					
++	1				1							13	5	4	3	1						
+	11			3	3	5						13	1	3	3	4	2					
±	16			5	7	4						9	1	1	2	5						
−	108			7	8	93						94	3		5	3	83					
TOTAL . . .	144											195										

Parasyphilis (*continued*)

Non-syphilitic												Total										
Wassermann	Number	Sachs-Georgi					Meinicke					Number	Sachs-Georgi					Meinicke				
		+++	++	+	±	−	+++	++	+	±	−		+++	++	+	±	−	+++	++	+	±	−
+++												335	262	37	24	8	4					
++												55	18	18	14	4	1					
+												72	6	7	18	24	17					
±												67	6	4	17	28	12					
−	189					189						693	6	4	26	30	627					
Total . . .												1,222										

Total		1,222
Results do not agree in 100 cases		8.1 %
Wassermann positive Sachs-Georgi »		495
Wassermann positive Sachs-Georgi negative		34 = 2.8 %
Wassermann negative Sachs-Georgi »		627
Wassermann negative Sachs-Georgi positive		34 = 2.8 %

In the second series of experiments we made 1,222 Sachs-Georgi reactions on a number of unselected cases. The proportion of non-concordant cases is 8%. 34 cases with positive Bordet-Wassermann reaction and negative Sachs-Georgi were found and 34 cases of negative Bordet-Wassermann reaction and positive Sachs-Georgi reaction. If the cases of slightly positive reaction are not taken into account, the percentage of non-concordant cases is decreased by half.

Consequently we can say that the concordance between these two methods is altogether remarkable. No better results are obtained with the fixation reaction by using varied antigens. The number of cases which give a positive Bordet-Wassermann reaction and a negative reaction with the Sachs-Georgi method is equal to the number of cases which give a positive Sachs-Georgi reaction and a negative Bordet-Wassermann reaction. We can say that our antigens afford an ideal degree of specificity.

By the use of the original extracts we obtained more divergent results. In Table IV is given the result of the tests in which we experimented simultaneously on the sera with antigen prepared by ourselves and that sent us by the discoverers (see Tables IV and V).

Table IV.

COMPARATIVE TEST WITH THE ORIGINAL SACHS-GEORGI EXTRACT AND THAT PREPARED IN WARSAW

		SG. Warsaw extract					SG. Original extract				
B. W.	Total	± ± ±	++	+	±	—	± ± ±	± ±	±	±	—
+++	471	355	64	34	14	4	316	65	28	19	43
± +	17		3	9	4	1		2	3	4	8
+	49	4	5	22	11	7	2	4	9	7	27
±	37	1	5	7	15	9	3	2	5	3	24
—	619	1	2	7	5	604	1	7	10	5	596
TOTAL	1,193	Results do not agree 36 = 3%					Results do not agree 125 = 10.5%				

A careful examination of the tables gives the following results:

For the Warsaw Extract :

BW. positive — SG. positive	563 = 96.5 %
BW. positive — SG. negative	21 = 3.5 %
	584 = 100 %
BW. negative — SG. positive	15 = 2.2 %
BW. negative — SG. negative	604 = 97.8 %
	619 = 100 %

In the case of the original extract :

BW. positive — SG. positive 482 = 82.5 %
BW. positive — SG. negative 102 = 17.5 %
584 = 100 %

BW. negative — SG. positive 23 = 3.7 %
BW. negative — SG. negative 596 = 96.3 %
619 = 100 %

Table V.

Comparative Test with the Warsaw Extract and the original Meinicke Extract

		Warsaw extract					Original extract				
B. W.	Total	+++	++	+	±		+++	± -	+	∴	—
+++	170	126	17	15	12		106	17	17	13	17
++	15		1	4	9	1			2	4	9
+	23		1	7	12	3		1		9	13
±	15		1		10	4				1	14
—	245					245					245
Total	468		Results do not agree 8 = 1.7 %					Results do not agree 53 = 11.3 %			

A careful examination of the tables gives the following results.

For the Warsaw Extract :

BW. positive — M. positive 215 = 96.4 %
BW. positive — M. negative 8 = 3.6 %
223 = 100 %

BW. negative — M. positive — 0
BW. negative — M. negative 245 = 100 %

In the case of the original extract :

BW. positive — M. positive 170 = 76 %
BW. positive — M. negative 53 = 24 %
223 = 100 %

BW. negative — M. positive — 0
BW. negative — M. negative 245 = 100 %

The tables show that the almost absolute coincidence which we found when using our own antigens was not manifested in the same degree when using original antigens. While the percentage of negative reactions with the Bordet-Wassermann method and positive reactions with the Sachs-Georgi method remain more or less the same, a considerable difference is seen in cases giving a positive Bordet-Wassermann reaction. Whereas with our antigen 3.5% of the sera giving a positive Wassermann reaction are negative with the Sachs-Georgi method, this figure reaches 17.5% with the original antigens. The Meinicke reaction gives an even greater difference. No negative Wassermann case gave a positive Meinicke reaction; but, on the other hand, the positive Bordet-Wassermann cases with our antigens gave 3.6% negative Meinicke reactions, and with the original antigens this figure reached 24%. These tests therefore show, on the one hand, the almost absolute specificity of the two methods, and on the other hand that the almost exact coincidence which we obtained is solely the result of the constitution and preparation of the antigen. The original antigens are therefore less sensitive than those of Warsaw. *By means of the precipitation methods we were able to make direct comparisons between the sensitiveness of the Wassermann reaction at Warsaw and at Heidelberg.*

You might ask, which is the most specific antigen? It is exceedingly difficult to reply to this question. We could prepare antigens which are as sensitive as they are rigorously specific by adding cholesterine according to the Sachs method and thus varying the precipitation property of the antigen.

In Table VI we have given the results obtained in accordance with the different stages of syphilis, and we have calculated the percentage of non-concordant cases for each stage (See Table VI).

Table VI.

Summary of Results obtained by the Sachs-Georgi Tests (3,563 cases)

	BW pos SG pos	BW pos SG neg	Percentage of results not agreeing	BW neg SG neg	BW neg SG pos	Percentage of results not agreeing	Total number
	—	—	—	—	—	—	
Syphilis I.	46	2	4	13	1	7	62
Syphilis II.	292	6	2	54	3	5.3	355
Syphilis III.	125	4	3	59	3	4.8	194
Latent syphilis	557	25	4.3	375	14	3.6	971
Parasyphilis.	33	1	3	10	1		45
Total number of cases of syphilis.	1,053	38	3.5	511	22	4.1	1,627
Non-syphilitic.	—	1	—	480	—	0	481
Doubtful	573	36	6	807	38	4.5	1,454

Summary of Results obtained by the Meinicke Tests (3,163 cases)

	BW pos. M pos.	BW pos. M neg.	Percentage of results not agreeing	BW neg. M neg.	BW neg. M pos.	Percentage of results not agreeing	Total number
Syphilis I	41	2	4.6	13	0	0	56
Syphilis II	245	2	0.8	36	0	0	283
Syphilis III	106	6	5.2	55	0	0	167
Latent Syphilis	517	45	8.0	368	3	0.8	933
Parasyphilis	33	1	3.0	10	1		45
Total number of cases of Syphilis	942	56	5.6	484	4	0.8	1,484
Non-syphilitic	0	1		0	480	0	481
Doubtful	441	49	10	690	6	0.8	1,186

The table shows that with our antigens we never found a positive reaction in a healthy man. The percentage of non-concordant cases was the same for the different stages of syphilis and in the doubtful cases. It is possible that the lower sensitiveness of original antigens may be due to the fact that the workers employed as controlling sera the sera of cancer patients (Sachs) or tubercular patients (Meinicke). We can therefore state that the flocculation methods provide us with an element of comparison (tertium comparationis) for the serological diagnosis of syphilis.

Can the precipitation methods, from the quantitative point of view, attain the intensity of the Wassermann reaction when it is positive?. We have established the results of our experiments in such a manner that the exact intensity of the complement fixation and flocculation is noted. In Table VII the result is established in such a way that the experiments corresponding to the fixation reaction are presented by the figure 100. The table includes 3,563 Sachs-Georgi cases and 3,163 Meinicke cases. It covers all the material used in our experiments, that is to say, treated and untreated syphilis, doubtful cases and control cases (see Table VII).

Table VII.

Comparison showing quantities between the sensitiveness of the Bordet-Wassermann, Sachs-Georgi and Meinicke Reaction with percentages.

	Wasser mann	Sachs-Georgi					Meinicke				
		+ + +	+ +	+	±		+ + +	+ +	+		
+ ±	100	*71*	15	0	5	1	*72*	11	10	6	1
+ +	100	5	*31*	*33*	20	10	5	17	*25*	*36*	16.6
±	100	4	5	*46*	34	11	3	2	*37*	*39*	18
±	100	1	5	17	*54*	23	2.9	3.9	11.8	33,6	*17.5*
—	100	0.2	0.6	0.7	1.9	*97.6*	0.2	0.2	0.18	0.18	*99.1*

This table shows that, out of 100 cases of strongly positive Wassermann reactions, only 71% of the flocculation reactions show a very strong precipitation, whereas 29% give a weaker flocculation. Out of 100 Wassermann cases ++, there are only 31% ++, 33%+ flocculation reactions. The remainder shows a more or less clear flocculation.

Out of 100 Wassermann cases+, and 100 Wassermann ±, about half the flocculation reactions present a corresponding degree of precipitation. Finally, out of 100 negative Wassermann cases, 97.6% of the flocculation reactions are negative and theremainder give varied positive reactions and even strong precipitations. The table therefore shows as a whole that the degree of the Wassermann reaction corresponds to the intensity of the flocculation reactions. There is, however, no absolute concordance, and there are cases in which the precipitation reactions are stronger and others in which they are weaker. If, therefore, we wish to take the degree of the Wassermann reaction as our standard, we cannot be sure of finding the same degree of intensity in positive reactions by a corresponding result in flocculation reactions.

The general table of cases studied according to the Meinicke method gives us slightly divergent results. 70% of the cases which are strongly positive with the Wassermann method are quite as strongly positive with the Meinicke method. But even when the Wassermann reaction is ++, the larger number of cases with the Meinicke method is only slightly positive and gives a low precipitation. When the Wassermann reaction is slightly positive, but all the same fairly clear, half of the cases examined with the Meinicke method are negative. The table also shows that when cases give strong positive reactions, the Sachs-Georgi and Meinicke reactions have the same value, but when the positive reaction becomes weaker, we find a much larger percentage of negative or doubtful Meinicke reactions than with the Sachs-Georgi method.

This in no way influences the principles of specificity of the reaction. The difference is due solely to the quality of the antigen and to the interpretation of the result. Although we consider that the two methods are equally specific, we prefer the Sachs-Georgi method, as it gives more complete parallelism with the complement fixation reaction and the slightly positive cases are recognised with greater certainty.

Some writers are of the opinion that in certain stages of syphilis flocculation reactions give better results than the Wassermann reaction. That is clear when more sensitive Sachs-Georgi antigens are prepared, but it is just the same for the Wassermann reaction. But if the antigens for the Sachs-Georgi reaction are carefully titrated, it will be impossible to obtain, at any stage of syphilis, different results with the method of complement fixation than those obtained with the precipitation method. In Table VI we have given the results of our cases with regard to different stages of syphilis; the non-concordant cases were calculated in %, especially in the case of positive Wassermanns, which, with the Sachs-Georgi method, became negative, or the contrary.

Our Sachs-Georgi antigen is the highest titration, since the number of non-concordant cases in both directions is more or less the same. We did not find any noticeable difference between the different stages of syphilis. The Meinicke extract is less sensitive; the table does not show that this reaction has any advantage over the fixation reaction. Looking over the reports of the cases under treatment, the same result is noted, that is to say that the intensity of the two reactions is in most cases identical, or else one or other of the methods gives more or less strong precipitations. No regularity is noticeable here (Table VI).

It appears that in some diseases there is no coincidence between the precipitation method and the complement fixation test. Thus we examined seven cases of relapsing fever which four times were negative with both methods; three times the Wassermann reaction was strongly positive; the Sachs-Georgi reaction was once slightly positive and twice negative.

In this case the Wassermann reaction was less specific. Out of 16 tuberculous sera, 2 gave positive Wassermann reactions and 14 negative reactions. Of the latter, two gave ++ reactions, one + and one ±. In 10 cases of negative Wassermann one gave a + Meinicke reaction and another a ± Meinicke reaction. In 16 cases of malaria, one serum gave a positive reaction with both methods and eleven gave negative reactions. On the other hand, three ++ Wassermann reactions and one + Wassermann reaction were negative with the Sachs-Georgi method. In these reactions the number of non-concordant cases is exceedingly high. In eight cases of typhus the results were identical with both methods We shall continue carrying out these experiments, but we should like to stress the fact that the difference in the two methods in certain diseases is not influenced by the specificity of the reaction if we know these affections and can exclude them. Moreover, it seems that in the case of malaria and relapsing fever, flocculation reactions give a greater specificity than the fixation reaction.

The following conclusions can be drawn from the results of our experiments :

(*a*) When the antigen is well tested, there is remarkable concordance between complement fixation reactions and Sachs-Georgi and Meinicke precipitation reactions (relapsing fever, malaria and tuberculosis have still to be studied).

(*b*) In two-thirds of the cases the intensity of the result of Bordet-Wassermann reaction corresponds to that obtained with the precipitation method. One-third of the cases sometimes react either more slightly or more strongly.

If *intensity* of the Wassermann reaction is taken to *estimate the degree of the disease*, we cannot be certain, in any given case, that this degree will be given with the precipitation method.

Dreyer Sigma Reaction

For reasons over which we had no control, until now we have only been able to examine 500 cases with the Dreyer method. These cases have also been examined with the Wassermann and Sachs-Georgi methods (original antigen IV). (See Table VIII.)

Table VIII.

Showing the number of Cases examined by the Dreyer and Sachs-Georgi Tests

Untreated syphilis I.	1
Untreated syphilis II.	4
Untreated syphilis III	—
Untreated latent syphilis	15
Syphilis under treatment	338
Total cases of syphilis.	358
Control.	61
Total	419
Doubtful cases.	81
Total of all cases	500

We followed the perfected technique which was sent us by the Chairman of the Health Committee in the name of Dr. Dreyer.

Certain difficulties were met with in calculating the units. The decrease of the precipitation intensity varies according to the different sera when dilutions of varying strengths are used. The number of units, calculations based on several tubes, is therefore different for the same serum. We do not wish to consider our positive cases again, but will only illustrate them by a few examples (see Table IX).

Table IX.

Summary of several positive Cases according to Dreyer Methods

	Tubes										
No.	2	3	4	5	6	7	8	9	B. W.	S. G.
3887	T	T	T	S—	S—	tr.	— —	—	+++	+++
4057	T	T	T	S—	S—	S.	tr—		++—	++—
3904	T	T	T	S	tr				++—	++—
3972	T	T—	S—	tr					++—	++—
3944	T	T	S—	S—	S	tr.			+	+—
3890	T	S	tr						+	++—
4020	T	tr	tr						+++	+++

The number of units calculated in the different tubes on a flocculation basis do not show, therefore, the intensity of the serum. If one takes the last tube as a guide and only reading the degree from it, the same values are not obtained as those given when the intensity of precipitation is taken into consideration.

If it is desired to calculate the number of units by taking the intensity of precipitation as a basis, a different interpolation formula should be applied to each serum. An important role is played in the precipitation reaction by two opposed phenomena: albumens possess a defensive power, and prevent the precipitation the reagents on the other hand are specifically favourable to precipitation. The intensity of the flocculation is due to the relation of these two powers, and it seems to vary in different sera. Taking this fact as a basis, it seems to us very difficult to apply a unique formula to all sera. However, in order to obtain some useful results, we calculated the number of units in the tubes giving a decreasing precipitation (generally three tubes) and then took the average. We were quite aware that this mathematical average should only be considered as a rough figure, especially as it must be remembered that Dreyer calculated the result in decimal units of fractions. To give a general idea of our results, we distinguished the following positive intensity of the reaction in our tables : altogether negative. 1 to 4 units (4), from 4 to 8 (8), from 8 to 25 (25), from 26 to 100 (100), from 100 to 250 (250), etc. In Tables X and XI the cases in different stages of syphilis are given.

Table X contains the number of units compared with the intensity of the Wassermann reaction. Table XI gives the comparison with the Sachs-Georgi reaction (original antigen). (See Tables X and XI.)

Table X.

Comparative Investigations regarding the Bordet-Wassermann reaction and the Precipitation method of Dreyer.

Syphilis I

	Cases treated							Cases untreated							
B. W.	Number	1,000	500	250	100	25	8	1	Number	1,000	500	250	100	25	8	1
+ + +	4			2	2				1			1				
+ +	1					1										
+																
±																
—	4							4								

Syphilis II

	Cases treated							Cases untreated							
B. W.	Number	1,000	500	250	100	25	8	1	Number	1,000	500	250	100	25	8	1
+ + +	6			1		4		1	4		3	1				
+ +	1						1									
+																
±	1						1									
—																

Syphilis III

B. W.	Cases treated: Number	1,000	500	250	100	25	8	4	—	Cases untreated: Number	1,000	500	250	100	25	8	4	—
+++	2			1		1												
++																		
+	1			1														
±																		
—	1								1									

Latent Syphilis

B. W.	Cases treated: Number	1,000	500	250	100	25	8	4	—	Cases untreated: Number	1,000	500	250	100	25	8	4	—
+++	96	2	5	16	26	27	9	1	10	15	2	3	8	1	1			
++	7		1				2	4										
+	19			1		3	6	6	3									
±	35				1	2	11	6	15									
—	160				2	2	2	13	141									

B. W.	Doubtful: Number	1,000	500	250	100	25	8	4	—	Without a clinical history: Number	1,000	500	250	100	25	8	4	—
+++	7		1	2			1		1	26		3	5	12	3	3		
++										3			1	2				
+	5							1	4	7			2		1	2	1	
±	1								1	5					2			3
—	11					2	4	2	3	16			1		2	1	2	10

B. W.	Control: Number	1,000	500	250	100	25	8	4	—	Total: Number	1,000	500	250	100	25	8	4	—
+++										161	7	13	36	43	36	13	1	12
++										12		1		1	3	3	4	
+										32			3		5	8	8	8
±										42				1	4	12	6	19
—	61								61	253			1		6	7	17	222

Table XI.

Comparative Investigation of the Flocculation Reaction according to Dreyer and Sachs-Georgi

(Tabulation of 500 cases without taking the clinical history into consideration.)

S. G.	Number	1,000	500	250	100	25	8	4	—
+++ —	144	3	11	28	40	24	21	9	8
±	49	—	3	9	11	10	7	6	3
. . . .	25			3	1	4	3	7	7
±	25			1		3	1	1	19
. . . .	257				1	2	5	7	242

The tables show the number of units in different stages of syphilis. It will be seen that Dreyer's method gives as specific

results as the other flocculation method. In order to state whether the degree of positiveness obtained by the Wassermann reaction on Sachs-Georgi corresponds to the number of Σ units, we calculated total results of our tests in percentages, in the manner that we determined, the number of cases corresponding to the intensity of B. W. or Sachs-Georgi with 100 (see Tables XII and XIII).

Table XII.

Comparison showing Quantities between the Sensitiveness of the Wassermann Reaction and the Precipitation Method according to Dreyer. (500 Cases) given in %.

	Wassermann	*Dreyer* — Number of units						
		1,000	500	250	100	25	8	4	—
+	100	4	8	22	27	22	8	0.6	8
+	100	—	8	—	8	25	25	33	—
+	100	—	—	—	9	16	25	25	25
±	100	—	—	—	2.5	9.5	28	14	45
— . . .	100	—	—	0.4	-	2.3	2.8	7	87.7

Table XIII.

Comparison showing Quantities between the Precipitation Method according to Sachs-Georgi and Dreyer, giving Percentages

	Sachs-Georgi	*Dreyer* — Number of units						
		1,000	500	250	100	25	8	4	
+	100	2	7.6	19	27.8	16.8	14.5	6.2	5.5
+	100	—	6.1	18	23	21	14	12	6
+	100	—	—	12	4	16	12	28	28
±	100	—	—	4	—	12	4	4	76
—	100	—	—	—	0.45	0.85	2	2.7	93.8

This table shows that when the Wassermann reaction is strongly positive the number of units can rise from 0 to 1,000. The greatest number of cases are between 100 and 250 units. When the Wassermann reaction is ++, half of the cases are from 8 to 25 units, but 33% have begun to react doubtfully. When the Wassermann reaction is + 50% the cases have begun to react negatively or give doubtful results with the Dreyer method. In 50% of the slightly positive Wassermanns we found 60% doubtful or negative Dreyer reactions. It is thus seen that Dreyer antigen gives specific reactions (it is very rare that the Dreyer reaction is positive when the Wassermann reaction is negative), but that its sensitiveness, when compared with the fixation reaction in practice in our Institute, is lower.

Table XIII shows that the antigen IV sent by Sachs is more sensitive than the Dreyer antigen. For example, in 100 Sachs-Georgi cases, 56 cases reacted doubtfully or negatively with Dreyer antigen and in 100 cases where the Sachs-Georgi reaction was ± 80% of the Dreyer reactions did not correspond to the diagnosis. The higher degree of sensitiveness of the Sachs antigen cannot be modified by any factor of sensitiveness, as it sometimes happens that Dreyer antigen gives clearer results. So as to compare the sensitiveness of the two antigens, two series of tables have been prepared, one with Dreyer antigen and the other with Sachs-Georgi antigen. At the end of 24 hours the two series were read in the Dreyer apparatus. Up till now a total number of 100 cases have been studied (see Table XIV).

Table XIV.

Comparative Investigation of the Sensitiveness of the Original Antigens of Dreyer and Sachs-Georgi

	Dreyer Antigen							*B. Wassermann*	*Sachs-Georgi Antigen*						
	3	4	5	6	7	8	9		3	4	5	6	7	8	9
3957	-	-	-	-	-	-	-	±	T	S—	-	-	-	-	-
3972	T	S—	tr	-	-	-	-	-	T	T—	S	S—	tr	tr	-
3271	-	-	-	-	-	-	-	-	S—	S	tr	tr—	-	-	-
3944	T	S—	S—	S	tr—	-	-	-	T	T—	S	-	-	-	-
3990	S	T	T—	T	S—	tr—	-	-	S	S	tr—	-	-	-	-
4206	T	T	T	S—	S	S	S	-	T	T	T—	S—	tr	tr?	-

In Table XIV we have collected a few cases where Sachs original antigen shows sometimes a greater sensitiveness and sometimes a lower sensitiveness than the original Dreyer antigen. In the majority of cases the Sachs antigen was more sensitive. We note, however, that there is a certain element of selection between the positive serum and the antigens: thus one serum reacts better with a certain antigen and another serum with some other antigen. Similar observations have been made in the Wassermann reaction. Two Serological Institutes can therefore note a varied number of units for the same serum, as it has been proved that an important role is played by the sensitiveness of the antigen to each serum.

To sum up, it can be said that the Dreyer method gives specific results. Original Dreyer antigen is a little less sensitive than that employed for our Wassermann reaction. It seems to us impossible to introduce a sensitive factor or to calculate the positive results in units, taking as a basis an interpolation formula applicable to all sera.

General Considerations.

The purpose of the different precipitation methods is really to render the antigen so labile that a flocculation may be observed macroscopically. This can be done in two ways :

(*a*) By the Meinicke method, by placing distilled water, etc. ;

(*b*) By adding cholesterine according to Sachs' method.

The Dreyer method aims at transforming the precipitation method by the addition of cholesterine so as to obtain quantitative results, which was very desirable.

The Dreyer method, therefore, cannot be opposed to the Sachs-Georgi method, but should be discussed from the point of view of a perfected technique of the fundamental method. We will examine, first of all, on their general lines, the Sachs-Georgi and Meinicke methods and then go on to the perfected technique of Dreyer. We have already established that the two methods give specific results; the results in cases of serious tuberculosis, malaria and relapsing fever, which are not very clear, hardly affect the perfect concordance of these two methods. In spite of their great specificity, we do not believe, however, that these two methods can, in their present state, replace the Wassermann reaction. It is easier to make a flocculation reaction for syphilis, but there are difficulties in reading the results, especially in the case of slightly positive results. We have often observed that the reading of the results depends on the individual variations of sensitiveness in the experimenter.

For instance, I am an astigmate and when I am very tired black always seems to me grey. I have observed the influence of secondary factors, such as fatigue and headache, both on myself and also on my colleagues; the capacity of appreciation is variable. In the fixation reaction these causes of error are excluded. We consider that the Wassermann reaction is superior to the flocculation reactions because it gives much clearer results than the latter. Of the two flocculation methods, we prefer the Sachs-Georgi or that of Meinicke. We would point out, however, that we do not wish to disqualify the Meinicke method, which gives good and specific results. We prefer the Sachs-Georgi method because the precipitations are manifested more clearly, and in cases where the positive Wassermann reactions decrease, the precipitation of the Sachs-Georgi decreases also, whereas the Meinicke method gives rapid reactions which are not clear.

Finally, by the addition of cholesterine the sensitiveness of the antigen can be varied in an easy and most convenient manner.

The Dreyer technique tries to utilise the principle of precipitation to estimate positive reactions quantitatively. Certain elements of the Dreyer reaction seem to us to show real progress. Dreyer advises that nine tubes should be employed with decreasing quantities of serum, as some sera, when too highly concentrated,

engender zones of restraint. We have come across sera with such small reaction zones that they only gave precipitations in one or two of the middle tubes. In practice we consider that it is sufficient to use three tubes with 0.1, 0.2 and 0.3.

Dreyer tries, with the help of an ingenious apparatus, to produce the dilution of the antigen mechanically. Although experienced experimentists can prepare satisfactory antigens by following Sachs' indications, it is advisable for inexperienced experimentists to prepare antigen with the help of an apparatus.

Dreyer advises that nine tubes should be taken for reading the degree of positive reactions. We think that this precision is unnecessary. The results of the Wassermann reaction can also be established more quantitatively. In spite of this, the fixation reaction, which is based on millions of cases, does not claim to give such rigorous precision (except in the case of cephalo-rachidian liquid). It does not attempt to do so because it is well understood that the *degree* of the Wassermann reaction helps us no longer from the point of view of diagnosis.

Our tables show that secondary syphilis gives on an average the strongest positive reactions, but we can draw no conclusions on the form and seriousness of the disease from these degrees of intensity of the reaction. Such precision does not seem necessary to us and we consider it cumbersome in the practical execution of sero-reactions in large Institutes.

As for the preparation of antigen, we can see no advantage in the manner of preparation advised by Dreyer; it is possible, however, that Dreyer's antigen keeps longer. Our experience gives us no indications in this matter. Original Dreyer antigen is less sensitive than Sachs antigen No. 4. This naturally does not effect in any way the principle of preparation, as by adding cholesterine, according to the Sachs method, we can vary the sensitiveness of the antigen. When reading the results, the agglutinoscope and Dreyer apparatus may be used. However, we prefer the Dreyer apparatus, as with the agglutinoscope one is obliged to diminish the light for each series of tubes as the clearness of the reaction depends on the intensity of the lighting. In the Dreyer apparatus, the same light comes always from above and the tube appears on the same background. We should like to propose that the apparatus should contain a band on which a series of tubes could be placed in one row, as it is difficult to take out each tube separately, either when the Sachs reaction is being made with the Dreyer method or the Sachs-Georgi method. It would also be more agreeable from the technical point of view if the Dreyer tube were wider.

To indicate exactly the intensity of positive reactions, Dreyer proposes that a calculation should be made giving the titre and the flocculation data in the form of one formula. Such a calculation would be justified for all sera if it gave the same precipitation in increasing dilutions. This is not the case, however, and one would therefore have to have a different interpolation formula

for each serum (1). From the practical point of view we consider this calculation unnecessary. When a laboratory gives one result + + and another +, when using good antigens, the doctor and the patient understand that these variations depend on the method and have no special significance. But when we give an answer in decimal fractions and another experimentalist gives an answer which differs by 50% from our own, we run the risk of completely discrediting methods of serological diagnostic. The lack of precision in the method cannot be remedied by mathematical descriptions.

In the same way, the variable sensitiveness of antigens cannot be expressed by a formula, as flocculation depends also on elective phenomena which cannot be translated into a formula. We should try by adding cholesterine to give the same sensitiveness to antigens, but we cannot make corrections with a factor of sensitiveness determined by a single serum. This is why we should put aside the calculation of the degree of positive reactions by units.

We have already insisted on the fact that these methods, on account of the difficulties in the reading of the results, cannot replace the Wassermann reaction. Since the preparation of more sensitive antigens renders the specificity of the reaction doubtful, there can be no question of rendering their constitution still more labil. On the other hand, the precipitation methods seem to me to be useful for comparing the sensitiveness of the Wassermann reaction in different Institutes. Our results given here contribute to such experimentation, as our Wassermann reaction showed greater sensitiveness than the precipitation methods carried out with the use of original antigens. We cannot find an identical expression, universal from the quantitative point of view, for the serological reaction of any one patient in all our laboratories, but we can reduce these differences to a minimum if all the laboratories regulate and standardise their Wassermann reactions by precipitation methods. The tubes introduced by Dreyer greatly facilitate this standardisation. It will be impossible to arrive at a state where a specific serum will give the same degree of flocculation in different Institutes, but by taking into consideration a series of experiments, it will be possible to attain relative standardisation.

With regard to the choice of a flocculation reaction we prefer that of Sachs-Georgi, carried out with the improvements introduced by Dreyer.

I believe that the following reply can be made to the question put by the Health Committee of the League of Nations to those who took part in the London Conference : We cannot replace the fixation reaction by flocculation reactions, but we can standardise the former to a certain degree by the use of the flocculation methods.

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(1) During the discussion at the Paris Conference, Dreyer stated that it is possible to explain this difference by the incomplete flocculation, which can be avoided. This subject will be discussed in the next report.

Protocol of Cases examined while under Treatment

No.	Diagnosis	Treatment	Date of examination	BW	SG	DM
—	—	—	—	—	—	—
4896	Syphilis II.	Hydr. Salv.	18/1			
		» »	10/2			
4897		Hydr. Salv.	18/1			
		» »	12/2			
		» »	15/3			
4899		Cal. Hydr. Salv.	18/1			
		Hydr. Salv.	15/2			
5190	»	Hydr. Salv.	20/1			
		» »	10/2			
		» »	1/3			
5186		Hydr. Salv.	20/1			
		» »	10/2			
		» »	1/3			
5191	»	Hydr. Salv.	20/1			
		» »	10/2		+	+ +
5185	Syphilis I.	Hydr. Salv.	20/1			
		» »	15/2			
		» »	1/3			
5192	Syphilis II.	Hydr. Salv.	20/1			
		» »	15/2			
5188		Bismut.	22/1			
		»	15/2		—	
5269		Bismut.	3/2			
		»	1/3		—	
5270	»	Hydr. Salv.	3/2			
		» »	1/3		—	
5271	»	Hydr. Salv.	3/2			
		» »	1/3			
5660	Latent syphilis	Hydr. Salv.	22/2			
		» »	18/3			
		» »	27/3		+	
		» »	12/4			
5661	Congenital syphilis	Hydr. Salv.	20/1			
	»	» »	20/2			
5741	Latent syphilis	Hydr. Salv.	24/2			
	» »	» »	22/3			
		Kal. Jod. Salv.	6/4			
	» »	» »	12/4			
5746	Syphilis II.	Hydr. Salv.	24/2			
		» »	22/3			+
		Kal. Jod. Salv.	31/3			
		Hydr. Salv.	6/4			
6186	»	Hydr. Salv.	10/3			
		» »	10/4			
6184		Hydr. Salv.	10/2			
		»	10/3			

No.	Diagnoses	Treatment	Date of examination	BW	SG	DM
6448	Lues tard.	Hydr. Salv.	6/3		+++	
	»	» »	31/3			
	»	» »	6/4	+++	+++	
	»	» »	12/4	+++		
5140	Syphilis II.	Hydr. Salv.	27/1	+++		
	»	» »	22/2		+	
	»	» »	1/3			
	»	» »	27/3	+—	+—	+—
4986	»	Hydr. Salv.	20/1			
	»	» »	27/1		++	
	»	» »	7/3			
5143	»	Hydr. Salv.	27/2			
	»	» »	7/3			
6245	»	Hydr. Salv.	13/3	+++		
	»	» »	31/3		+++	+++
	»	» »	6/4		+++	+++
	»	» »	12/4	+++	+++	
5144	Syphilis lat.	Hydr. Salv.	27/1		+++	
	»	» »	7/3	+++		
6242	Syphilis I.	Hydr. Salv.	13/3		+++	+++
	»	» »	31/3			+++
5315	Syphilis latent.	Hydr. Salv.	3/2	+++		
	» »	» »	1/3	+++	++	
	» »	» »	10/3	+	+	+—
5318	» »	Hydr. Salv.	3/2	+++	+++	
	» »	» »	17/2		+++	
4708	» »	Hydr. Salv.	11/1	+++	+++	
	» »	» »	3/2			
5323	» »	Hydr. Salv.	3/2		++	
	» »	» »	7/3			
5328	» »	Hydr. Salv.	3/2			
	» »	» »	3/3			
	» »	» »	23/3			
4713	» »	Salv.	3/2			
	» »	»	27/2	+++		+++
	» »	»	23/3			
5747	Syphilis III.	»	27/2	+++	+	
	»	»	3/4			
6073	Syphilis II.	Hydr.	3/3			
	»	»	22/3		+++	
	»	Bism.	4/4	+++		+++
	»	Salv.Bism.				
6839	»	Salv.	23/3	+++	+++	+++
	»	»	27/3		++	
	»	»	3/4			+++
6281	»	Salv.	10/3			+++
	»	»	1/4	+++		+++
	»	»	10/4			+++
6284	»	Salv.	22/3			+++
	»	»	27/3			+++
	»	»	4/4	+++		+++
	»	»	10/4		+	+
6831	»	Salv.	22/3		+++	+++
	»	»	27/3		+	+++
	»	»	4/4		+++	+++

No.	Diagnoses	Treatment	Date of examination	BW	SG	DM
6482	Syphilis II.	Hydr.	23/3	− + −	+ + +	+ − −
	»	»	31/3	+ + −	+ + +	− + −
	»	»	12/4	+ + +	+ + +	+ + +
1003	»	Bism.	23/5	+ + −	+ + +	+ + −
	»	»	6/6	+ + −	+ + +	+ + +
	»	»	29/6	+ + +	+ −	−
704	»	Bism.	29/5	− + +	− + +	+ + +
	»	»	6/6	− + −	− + −	− + −
437	Syphilis III.	Bism.	18/5	+ +	+ +	+
	»	»	6/6	+ −	+	−
	»	»	16/6	+ +		−
436	Syphilis II.	Bism.	18/5	+ + −	− + +	+ − −
	»	»	29/5	+ + +	+ + +	+ + +
	»	»	6/6	+ + +	+ + +	+ + +
1009	Syphilis latent.	Hydr. Salv.	18/5	+ + +	+ + +	+ + −
	» »	» »	6/6	+ + +	+ + +	+ + +
1007	» »	Hydr. Salv.	22/5	+ + +	+ + +	+ + +
	» »	» »	6/6	+ + +	+ + +	− + +
	» »	» »	19/6	− + +	+ +	+ +
6953	Syphilis III.	Hydr. Salv.	23/3	+ + +	+ + +	+ + −
	»	» »	28/4	− + −	+ + +	+ + +
6950	Syphilis II.	Hydr. Salv.	23/3	+ + +	+ + +	+ + +
	»	» »	28/4	+ + +	+ + +	+ + +
6947	Syphilis I.	Hydr. Salv.	23/3	− + +	− + +	+ + +
	»	» »	28/4	—	—	−
6946	Syphilis II.	Hydr. Salv.	23/3	+ + +	− + +	+ + −
	»	» »	28/4	+ + −	+ + +	+ + +
6945	»	Hydr. Salv.	23/3	+ + +	+ + +	+ + −
	»	» »	28/4	+ + +	+ + +	+ + +
6482	Syphilis II.	Hydr. Salv.	23/3	+ + +	+ + +	+ + +
	»	» »	31/3	+ + +	+ + +	+ + +
	»	» »	28/4	+ + +	+ + +	+ + +
108	»	Bism.	22/4	—	—	—
	»	»	1/5	+ + +	− + +	+ + +
	»	»	6/6	+ + +	+ + +	+ − +
115	»	Hydr. Salv.	24/4	− + +	+ + +	+ + +
	»	» »	6/5	− + +	+ + +	− + −
7500	»	Hydr. Salv.	24/4	+ + +	+ + −	− + +
	»	» »	6/5	− − +	+ + +	− + −
6092	»	Hydr.	8/3	+ − +	+ + +	+ + −
	»	Hydr. Salv.	28/4	+ + +	+ + +	+ + +
6094	»	Hydr.	8/3	+ + +	+ + +	+ + +
	»	»	28/4	+ + +	+ + +	− + +
7395	»	Bism.	20/4	+ + +	+ + +	− + −
	»	»	28/4	+ + +	+ + +	+ + −
	»	»	6/5	+ + +	+ + +	+ + +
5321	»	Bism.	20/4	+ + +	+ + +	+ + +
	»	Hydr.	19/6	+ + +	+ + +	− + +

A COMPARATIVE STUDY OF THE FLOCCULATION REACTIONS OF MEINICKE (S. M.) SACHS-GEORGI AND THE ORIGINAL WASSERMANN REACTION

By Professor W. Kolle, Frankfort

Between January 1st and October 19th, 1922, the following cases were examined :

894. Untreated cases of syphilis.

749. Treated cases of syphilis.

578. Non-syphilitic cases, or sera from persons with no history of syphilis.

Only cases with undoubted clinical history were dealt with. As previous investigations had established the fact that sera with "strong positive" Wassermann reactions regularly gave also positive flocculation, only a few strong positive sera were included in the series as controls.

It was then necessary to determine whether the flocculation test would also correspond to the results obtained with the W. R. when using "weak" positive W. R. sera as well as cases giving only "traces" in W. R. For this reason, for the most part cases of this nature were examined (early stages, latent syphilis, metasyphilitic diseases, etc.).

Table

	Number of cases examined	Wassermann		Indications ++ with 1/20 complement +	Sachs-Georgi		Indication	Meinicke		Indication
		+	−		+	−		+	−	
Untreated cases of syphilis . .	894	375	440	79	350	523	21	355	522	17
Treated cases of syphilis. . . .	749	237	445	67	199	525	25	198	531	20
Cases with no clinical evidence of syphilis.	578	22	553	3	23	554	1	19	559	
Total. . .	2,221	634	1,438	149	572	1,602	47	572	1,612	37

The Wassermann reaction was carried out in accordance with the directions laid down by the State Health Department (*Reichsgesundheitsamt*).

As regards the conclusion which may be drawn from these investigations, the figures speak for themselves. A marked simi-

larity exists between the results of the flocculation and the Wassermann reactions. I consider the use of *both* the flocculation reactions side by sid as important, for the reason that, in the case of the Meinicke reaction, non-cholesterinised extracts and, in the case of the Sachs-Georgi reaction, cholesterinised extracts are used. Moreover, by applying two flocculation reactions besides the Wassermann reaction, we can obtain a better idea as to whether the case is one of syphilis or not, because the congruity of two reactions (*e.g.*, Wassermann positive, Sachs-Georgi positive, Meinicke negative) affords a more reliable result than if a flocculation reaction alone were used. It may occur then that the Wassermann reaction, for example, may be positive and the Meinicke negative. In this case a third reaction would be performed, in order to test the positive result of the Wassermann reaction, or, if the reverse were the case, of the flocculation reaction.

COMPARATIVE INVESTIGATIONS ON THE FLOCCULATION TESTS OF DREYER, MEINICKE AND SACHS-GEORGI

By Dr. Rudolf Müller, Vienna.

Introduction.

The theme proposed to us by the Health Committee of the League of Nations is the following :

Comparative investigations of the three flocculation methods of Dreyer, Meinicke and Sachs-Georgi, between one another and towards the Wassermann reaction.

The use of the original technique and of original extracts is imposed for the flocculation tests, whereas no uniform method is prescribed for the performance of the W. R. but the technique ordinarily in use at the respective station. This condition is, no doubt, founded on the consideration that the less complicated flocculation methods, applied with a uniform extract, should give identical results, and that in this way the important verification of the different modes of performance of the W. R., as to their comparative value, would be obtained. But, even at the beginning of our investigations, we had to realise that a perfect quality of the extracts can in no case be relied upon, even when the latter are delivered from the most competent sources. This may be inferred from the following fact: We have for some years used the Meinicke reaction in our laboratory, partly with original extracts and partly with extracts prepared by us, and we could in most cases verify a most pronounced homogeneity of the extracts. But precisely the original Meinicke extracts (24, 25, 26 and 27) received for the accomplishment of the present investigations were partly of considerably lesser efficiency, so that we should never have obtained results answering to the spirit of our task if we had employed these extracts exclusively. If we desired to reach our purpose, it was compulsory to use extracts prepared in our own Institute as well. Neither were the original extracts delivered by Sachs always completely satisfactory. An alteration of the extracts may have been produced by their being posted. The following fact, together with other facts, would tend to witness in this sense: Some of the original extracts, *i.e.*, extract XXXV and the first sample of II, proved quite impossible of employment; both of these extracts had been sent to us in January, at the time of the severest cold. Other samples, again, showed different results in our Institute, when compared between themselves, than in the Sachs Institute.

For instance : the comparative testing of the extracts II and IV, received in April, showed stronger results for the former extract, whereas, according to a letter from Sachs, the latter should be the stronger. From Dreyer we received a unique and efficient extract.

The idea that such fortuitous circumstances must be of influence on comparative researches, and the reflection that a small variation in the technique (see, for example, lengthened period of incubation, as suggested by Dreyer) could strongly lessen the value of the results of this year's work, have induced me to examine questions of theoretical importance on the occasion of the study of the imposed theme. The fact that only the main, but not all, flocculation methods (Bruck, Dold, Heckt, Kalin, Vernes, etc.) were put within the range of our investigations has influenced us in the same direction.

In my opinion, the first thing would be to try to analyse the empirically found tests on chemical and colloid-chemical principles. In this way we will become acquainted with the reasons for the peculiarities of each method, compare the particular importance of each condition, and so approach as near as possible to the desired purpose : obtain a uniform method of flocculation, uniting the advantages of each of the methods proposed up to now.

In the second part of this paper we have tried to sum up shortly the essential differences between the three methods, and some results of colloid-chemical investigations, which we have undertaken in the above-mentioned spirit, are also recorded there.

Part I

Technique.

For technical details we followed as nearly as possible the original instructions for the flocculation tests. Nevertheless, small changes occasionally proved necessary, and we shall now proceed to give some details thereof.

We must not omit to mention that on some days, for reasons unknown to us, one or other of the precipitation methods gave bad results, either by all coming out too weak or by the control sera presenting flocculation.

1. *Sigma Reaction.* — In some cases, where the serum provision was insufficient, we had to renounce the Alpha reaction. In such cases the result would sometimes not be quite clear, *i.e.*, tube 2 would not show at least a flocculation corresponding to degree 1.5; then some more blood was taken from the patient and the Sigma reaction undertaken with the Alpha extract as well. We do not think we have unfavourably influenced, through this partial renouncement of the Alpha reaction, the total result of the Sigma reaction, for it is sometimes possible to ascertain a distinct reaction with the Alpha extract *alone;* this reaction will in most cases be easily distinguished from true precipitation by its optic effect, but not always with sufficient distinctness and with a precision allowing

the admission, from a positive Alpha reaction *alone* (without any reaction in one of the other tubes), of the diagnostic : syphilis.

Time of Readings. — As results entered in our table we chose chiefly the readings after 20-22 hours. We were able to do this because we had made readings after 20 hours as well as after 7 hours, even before the Dreyer method was complete in that direction. Readings after 40-44 hours were, however, only taken in some of the cases, as the communication concerning the lengthening of the duration of the test reached us rather late. We thought it necessary to proceed to calculations after comparison with control tubes only when the question arose as to whether a reaction should be counted as positive with an intensity of 1.5 units or as negative. Even in comparing with the standard tube, it was not in all cases easy to decide this point. There were cases, especially after 40-44 hours, where a definite decision was impossible, so that it was necessary to proceed to a repetition. We must also mention here that, although the Dreyer technique is sufficiently exact to render *great* differences between researches on different days very rare, there nevertheless appear, for reasons unknown to us, in some series of reactions somewhat stronger or weaker results than in others. We esteem it necessary to point out such irregularities, although these differences never attain, in Dreyer's method, such importance as to interfere seriously with the result of the investigation, for the value of an *exact calculation,* in ciphers, as proposed by Dreyer, is dependent on an *absolute* constancy in the results of reactions. If such an absolute constancy cannot be obtained, it would be best to accept for the Sigma reaction too the simple qualifications of positive and negative, with the intermediate degrees "incompletely positive" and "traces".

Water-bath. — It seems, judging from our comparative investigations, that no noticeable difference exists after 24 hours between readings on tubes treated by incubation and tubes held in the water-bath. Such a difference is only evident in incubations of short duration.

Conservation of Sera. — We could not observe flocculation caused by infection. We think it cannot be excluded that such a possibility is greater when the sera are received in test glasses containing some agar, as is sometimes done in order to facilitate the separation from the clot.

2. *Meinicke Reaction.* — The results of the Meinicke method concern experiments with extracts prepared by us, because the original extracts that were offered us gave much too weak results. The preparation was made exactly according to Meinicke's recommendations. From many extracts which we had prepared we selected those which proved the best when applied to a great material. The reaction was done with 2, 4 and 8 drops of serum. The results were read after a total of 24 or 48 hours, about 18 hours being counted for incubation. Cases that gave a distinct reaction only with 2 drops, and a negative or just noticeable positive answer

with 4 drops, were considered as negative (see Part II of this paper). Likewise, those cases have to be looked on as negative which showed very slight traces of flocculation after 48 hours and only in the 8-drops tube. A feeble reaction of this type can occasionally be observed with non-syphilitic sera.

A previous inactivation was not necessary, as we were able to convince ourselves that it had no essential influence on the degree of reaction.

3. *Sachs-Georgi Reaction.* — We used the original extracts sent to us by Sachs. We did not try our own extracts, notwithstanding the relatively weak reactivity of the original extracts, as no essential differences existed between the two sorts of extracts. We could not ascertain the cause of these relatively unsatisfactory results. It is possible that the cholesterol used by us was inappropriate. We believe ourselves authorised to say that the original extracts did not possess the normal efficiency, in that we previously obtained better results with the Sachs reaction than now. The results were to a certain extent accentuated by extending to 5 minutes the time between the first and second adjunction of saline solution when proceeding to the dilution of extracts. The reading was made, as prescribed, after 18 hours' incubation. We believe, nevertheless, that the Sachs method would also necessitate a longer stay at the room temperature, so as to detect syphilitic cases with only slight reactions. We proceeded to this reading only for our own orientation, but did not use it in our tables, in consideration of the well-known possibility of unspecific reactions. A longer experience of the subject is necessary in order to clear up this point. The recognition of slight Sachs reactions appeared to us somewhat more difficult than in the other flocculation methods. A more intimate experience of the Sachs method is necessary.

4. *Wassermann Reaction.* — We will take into view those principal points of the method made use of which we believe are the chief cause of many differences between our results and those obtained by the original technique.

(a) *Antigen.* — The preparation is shortly as follows : 10 gr. of finely chopped ox-heart are extracted by 100 cc. alcohol during 2 hours at 56 degrees and 24 hours at the room temperature. Filtration through paper filter. It is better to make use of such a stock extract not earlier than after 1-2 weeks.

Preparation of Antigens ready for Use. — 20 ccm. stock extract plus 3 cc. of a 1/2 % solution of cholesterol in alcohol, are reduced to a volume of 8 cc. in a water-bath of 100 degrees, in a broad graduated test-tube of about 2 1/2 c. diameter. These 8 cc. are then poured into 5 cc. of physiological saline solution which has been held ready in a glass. To this mixture is quickly added a further 20 cc. of the physiological saline solution. The so-prepared colloid solution is then held in closed tubes for 24 hours at

56 degrees. During this time the liquid precipitates. Just before beginning the reaction the suspension is shaken; the dispersion of the particles is very easy to obtain.

(b) *Complement.* — One does not proceed to a real filtration of the complement, but always works with the same quantity of complement. But it is essential to test the efficiency of the complement, especially in controlling the lack of fixation in non-syphilitic cases, and in excluding complements that prove unsatisfactory in this preliminary test.

(c) *Controls.* — Accessory to the usual controls we make use of an alcohol control (in place of antigen), as here and there a positive reaction is simply to be interpreted as a consequence of the presence of alcohol, and this result cannot be regarded as characteristic for syphilis.

(d) *Inactivation.* — We test as well active sera as sera inactivated during 1 1/2 hours at 56 degrees. The opinion must at least be revised that making use of active serum brings about a special increase of unspecific reactions. This opinion cannot be maintained if *correct quantities* of patients' sera be used and if our method is adopted. On the contrary, we must insist on the fact that, as a whole, inactivated sera give stronger results (provided always that our method be followed).

(e) *Other Details of Technique.* — We must forego further details of technique here and shall merely point to the corresponding publications. We shall only point out, as characteristic of our method, that we do not proceed to a separate dilution of the reagents, but begin by putting the whole quantity of saline necessary for dilution into the test-tubes and add the reagents in a concentrated state; as a unit of mass we chose one drop.

(f) *Readings.* — The active method as well as the inactive necessitate at least two readings. The choice of the right moment for the reading is of special importance as to the results. Each reaction renders numerous controls absolutely necessary. The first reading, as well for the active as for the inactive test, is made when a complete dissolution is realised in the normal controls. This moment depends partly on the strength of the haemolytic components made use of on the test day. It is not possible, as far as our opinion goes, to give a uniform incubation period, for this period will always have to be based on the dissolution of the controls. The first reading of the controls of the inactive test always comes later than in the active test, because of the lack of auto-complement. The second reading is made, after about half-an-hour, at the room temperature for the active test; for the inactive test it is made after many hours in the ice-house. If we want to avoid simply schematic data, but want to attain results that can be put into practice, it is necessary to separate first two sorts of cases : cases of syphilis where the test is undertaken to elucidate purely therapeutic questions, and cases where syphilis has to be ascertained by the reaction itself (diagnostic reactions).

In the latter group we have again to distinguish the interpretation of cases presenting a predominance of general symptoms (general infection, cancer, pregnancy, etc.) from cases which are healthy or suffering from slight local affections. It is only in this way that it is possible to detect really the positive reaction of syphilis and to avoid disturbing unspecificities. In cases where there is complete lack of data it is, of course, impossible to avoid a certain number of results classed as "dubious".

The following tables were prepared only with the intention of presenting in a clearly arranged manner the different combinations of positive and negative results. The vertical columns show the variations of the Wassermann and Meinicke, the horizontal ones the variations of the Sigma and Sachs-Georgi.

Examples. — Question : How many cases of latent syphilis are positive only with Sigma? (table IV, vertical column 3, horizontal 4). Answer : 10.

Question : How many cases of syphilis under treatment are positive only with the Wassermann and the Meinicke? (Table VIII, vertical column 1, horizontal 3). Answer : 33.

Table I.

Prim. syph.	S.-G.+ Dr.+	+ —	— —	— +
Wa.+M.+	24		4	4
Wa.+M.—	3	2	4	8
Wa.—M.—	1	1	32	3
Wa.—M.+			2	

Number of cases	88		
Wa. pos.	49	=	55.6%
Dr. pos.	43	=	48.6%
M. pos.	39	=	38.6%
S.-G. pos.	31	=	35.2%

Wa.	Dr.	M.	S.-G.
100	87.7	69.4	63.2

Table II.

Fresh Syph. II	S.-G.+ Dr.+	+ —	— —	— +
Wa.+M.+	52			
Wa.+M.—			1	
Wa.—M.—				1
Wa.—M.+				

Number of cases	54		
Wa. pos.	53	=	98.1%
Dr. pos.	53	=	98.1%
M. pos.	52	=	96.3%
S.-G. pos.	52	=	96.3%

Wa.	Dr.	M.	S.-G.
100	100	98.1	98.1

Table III.

Syph. III.	S.-G.+ Dr.+	+ —	— —	— +
Wa.+M.+	21	1	1	1
Wa.+M.—				
Wa.—M.—			1	
Wa.—M.+				

Number of cases	25		
Wa. pos.	24	=	96.0%
Dr. pos.	22	=	88.0%
M. pos.	24	=	96.0%
S.-G. pos.	22	=	88.0%

Wa.	Dr.	M.	S.-G
100	91.7	100	91.7

Table IV.

Syph. lat.	S.-G.+ Dr.+	+ —	— —	— +
Wa.+M.+	158	1	8	17
Wa.+M.—		1	27	29
Wa.—M.—	3		203	10
Wa.—M.+	4		7	8

Number of cases	476		
Wa. pos.	241	=	50.6%
Dr. pos.	229	=	48.1%
M. pos.	203	=	42.6%
S.-G. pos.	167	=	35.0%

Wa.	Dr.	M.	S.-G.
100	95.0	84.2	69.3

Table V.

Tabes	S.-G.+ Dr.+	+ −	− −	− +
Wa.+M.+	17	1		2
Wa.+M.−		2	5	1
Wa.−M.−			11	
Wa.−M.+				

Number of cases	39		
Wa. pos.	28	=	71.8%
Dr. pos.	20	=	51.3%
M. pos.	20	=	51.3%
S.-G. pos.	19	=	48.7%

Wa.	Dr.	M.	S.-G.
100	71.4	71.4	67.8

Table VI.

D. p.	S.-G.+ Dr.+	+ −	− −	− +
Wa.+M.+	8		1	1
Wa.+M.−				
Wa.−M.−				
Wa.−M.+				

Number of cases	10		
Wa. pos.	10	=	100%
Dr. pos.	9	=	90%
M. pos.	10	=	100%
S.-G. pos.	8	=	80%

Wa.	Dr.	M.	S.-G.
100	90	100	80

Table VII.

Syph. congenit. (Kerat. par.)	S.-G.+ Dr.+	+ −	− −	− +
Wa.+M.+	11	1	1	2
Wa.+M.−			2	
Wa.−M.−			5	
Wa.−M.+				

Number of cases	22		
Wa. pos.	17	=	77.3%
Dr. pos.	13	=	59.9%
M. pos.	15	=	68.2%
S.-G. pos.	12	=	54.5%

Wa.	Dr.	M.	S.-G.
100	76.5	88.2	70.6

Table VIII.

Syph. under treatment	S.-G.+ Dr.+	+ −	− −	− +
Wa.+M.+	271	7	33	49
Wa.+M.−	23	2	67	63
Wa.−M.−	4	3	102	12
Wa.−M.+	7		1	9

Number of cases	653		
Wa. pos.	515	=	79 %
Dr. pos.	438	=	68.0%
M. pos.	377	=	57.7%
S.-G. pos.	317	=	48.5%

Wa.	Dr.	M.	S.-G.
100	85.0	73.4	61.5

Table IX.

Total syphilis	S.-G.+ Dr.+	+ −	− −	− +
Wa.+M.+	562	10	49	76
Wa.+M.−	26	7	106	101
Wa.−M.−	8	4	354	26
Wa.−M.+	11		10	17

Number of cases	1,367		
Wa. pos.	937	=	68.6%
Dr. pos.	827	=	60.5%
M. pos.	735	=	53.7%
S.-G. pos.	628	=	45.9%

Wa.	Dr.	M.	S.-G.
100	88.2	78.4	670.

Table X.

Aortitis	S.-G.+ Dr.+	+ −	− −	− +
Wa.+M.+	5			4
Wa.+M.−			1	
Wa.−M.−			8	
Wa.−M.+				

Number of cases	18
Wa. pos.	10
Dr. pos.	9
M. pos.	9
S.-G. pos.	5

Table XI.

Ocular diseases	S.-G.+ Dr.+	+ −	− −	− +
Wa.+M.+	8			1
Wa.+M.−				
Wa.−M.−	1		54	
Wa.−M.+	1		3	1

Number of cases	71		
Wa. pos.	9	=	12.7%
Dr. pos.	12	=	16.9%
M. pos.	14	=	19.6%
S.-G. pos.	10	=	14.0%

Table XII.

Other diseases	S.-G.+ Dr.+	+ −	−− −	− +
Wa.+M.+		1	1	1
Wa.+M.−			4	3
Wa.−M.−	2	2	212	8
Wa.−M.+	1		5	1

Number of cases	241
Wa. pos.	10
Dr. pos.	16
M. pos.	10
S.-G. pos.	6

Healthy persons or patients suffering mild local affections : 340 cases, all negative.

Summary of Results

	Number of cases	Wa. R. pos.	Wa. R. neg.	M. pos.	M. neg.	S.-G. pos.	S.-G. neg.	Sigma pos.	Sigma neg.
Syphilis I untreated. .	88	49	39	34	54	31	57	43	45
Syphilis II untreated. .	54	53	1	52	2	52	2	53	1
Syphilis III untreated .	25	24	1	24	1	22	3	22	3
Syphilis lat.	476	241	235	203	273	167	309	229	247
Tabes untreated. . . .	39	28	11	20	19	19	20	20	19
D. p. untreated. . . .	10	10	—	10	—	8	2	9	1
Syph. cong. untreated and keratitis parenchym.	22	17	5	15	7	12	10	13	9
Syphilis under treatment.	653	515	138	377	276	317	336	438	215
Total syphilis.	1,367	937	430	735	638	628	739	827	540
Doubtful syphilis (aortitis and diseases of the eyes)	89	19	70	23	66	15	74	21	68
Other diseases (including some cases of uncertain diagnosis) . .	241	10	231	10	231	6	235	16	225
Healthy persons or patients with mild local affections.	340	—	340	—	340	—	340	—	340

Total of all cases : 2,037.

NOTES ON THE RESULTS RECAPITULATED IN THE TABLES

Syphilis I, II, and Paralysis (Tables II, III, VI).

The result of our comparative investigations, as reproduced in the tables, shows a pronounced parallelism only for syphilis II

and III and for paralysis. The equivalent of methods is much better when we consider latent syphilis (Table IV), under which term we mean cases free from symptoms, that have had no treatment for at least many months. The results of Dreyer and Wassermann were most equal here. This concordance is, however, only apparent in the total; if we compare one case with the other, we find, as can be seen from the table, 37 cases positive with W. and negative with Dreyer, and 25 cases presenting the inverse distribution. On the whole, there are consequently 62 cases giving contradictory answers, that is 12.8% of the total. This difference is much greater with the other reactions of precipitation, and I would insist once more upon the fact that we accomplished the M. R. mostly with extracts prepared by ourselves, because of the weakness of the original extracts; and upon the fact that the results given by S.-G. extracts remained below the results obtained in previous experiments. In spite of this inferiority of the M. R. as compared with the W. R., we find some cases still that were positive only with M. but not with W.; we find such cases even in the weakest of all tests : the S.-G. test.

Syphilis under Treatment (Table VIII).

The differences appear mostly in comparing cases of syphilis during the treatment. It is not to be wondered at that we verify here the greatest differences, as we have partly the weakest reactions of all, but some other causes seem to come into play. At any rate, we noticed in this group of cases that sometimes a completely negative result of the precipitation tests contrasted with a strongly positive W. R. Here and there the contrary can be found. This fact is not arrived at by chance, as we could often convince ourselves that, starting from a strongly positive result of all tests at the beginning of the cure, one after the other of the flocculation tests became negative, at a time when the Wassermann was relatively little changed. We have the impression that the process of treatment played a part therein, because we observed such great differences much more rarely in latent cases, where treatment had ceased some months previously. If in such cases there existed a divergence between W. and flocculation tests, the positive result was in most cases an incomplete one.

The Sigma test, in this group of cases under treatment, remains more behind the W. R. than in the latent group, but nevertheless, of all the precipitation tests, it approaches the W. R. most nearly.

In comparing the different cases we find :

Wassermann and Dreyer both positive	406
» » » negative	106
Wassermann pos., Dreyer neg.	109
» neg. » pos.	32
In consequence : contradictory cases	141

= 21.6% of the total.

Here also the two remaining precipitation tests remain behind. But it must be emphasised that, rarely it is true, one of these two tests can be positive, whereas the W. R. and Sigma R. fail. In spite of the rarity of the fact, such results cannot be regarded as fortuitous: effectively we obtained the same results in repeating the experiment.

Untreated Primary Syphilis (Table I).

The sero-reaction in primary syphilis has a special importance in its relations to the success of an abortive cure started in time. The table shows here also a pre-eminence of the W. R. If we consider again the diverging cases, we find :

Wassermann and Dreyer both positive . . .	39	cases
» » » » negative. . .	35	—
Wassermann positive, Dreyer negative. . . .	10	—
» negative » positive	4	—

That is : 14 not corresponding cases = 15.9% of the total. Here also the two remaining reactions of precipitation, which are more rarely positive, here and there come out positive; this happens twice for the Meinicke R. and once for the Sachs-Georgi R.

Tabes (Table V).

In tabes we find a marked pre-eminence of the W. R., to that extent that not one of tabes cases came out positive in one of the precipitation reactions that did not show also a positive Wassermann R. The three flocculation tests gave fairly the same results, each one of them being negative in the same number of cases, the W. R. being positive.

Congenital Syphilis (Table VII).

In 22 cases of congenital syphilis, especially keratitis parenchymatosa, the W. was again the reaction most often positive, and there was no case where the W. R. was negative whilst any one of the precipitation tests gave a positive answer.

General Results in Syphilis (Table IX).

About 1,367 cases of syphilis were submitted to testing. We would point especially to the general result shown by the table, because here the superiority of the W. R. shows itself most clearly. We shall discuss later on the conclusions to be drawn from this fact in what regards the diagnostic and therapy.

Aortitis and Ocular Diseases (Tables X and XI).

This group of cases — which we have catalogued in the general table as "Doubtful Syphilis" (aortitis and diseases of the eyes), because, as a matter of fact, syphilis is often admitted as a frequent etiology, but, on the other side, it is often impossible to decide, on clinical grounds, whether a given case has been caused by syphilis or by any other cause — takes in quite a special position; 18 of such cases of aortitis were examined. But from these only 3 cases had an anamnesis establishing syphilis in a peremptory way; some other cases were not plain; most of the cases lacked any elements pointing to syphilis. We must remark here that the 3 cases with positive anamnestic data were all positive by the W. R. and that, of the precipitation tests, the Sachs-Georgi failed twice, the Meinicke and Dreyer coming out negative in one case also. Only 7 of the remaining 15 cases had a positive W. R. The results obtained by sero-reactions in all the other types of syphilitic diseases will perhaps authorise the conclusion that the clinician's deduction of a syphilitic etiology in the said cases was not entirely exact.

In paying attention to our results in ocular diseases we arrived at similar views. The material examined consisted in : iritis, irido-cyclitis, many cases of scleritis, etc. In all these cases the clinical reports mentioned that there could not be found characteristic differences between syphilitic and non-syphilitic diseases. Papulous iritis was sorted with secondary syphilis, keratitis parenchymatosa with congenital syphilis, and the ocular diseases of tabes with the latter. Now we find that the great majority of these cases was negative in all tests. On the other side, it is precisely in this sort of diseases that the reactions of precipitation are more often positive than the complement binding reaction. It is all the more difficult to find an explanation for this remarkable fact as neither the anamnesis nor the result of therapy gave any clue. The 8 cases which were positive in all reactions were : twice choroiditis, once iritis, three times irido-cyclitis, once a spasm of convergency, and once opacities. One irido-cyclitis was positive exclusively with Sachs-Georgi and Dreyer; another irido-cyclitis positive in all 3 precipitation tests but negative with W. R. One scleritis was positive in all reactions excepting S. G.; an iritis only positive in M. and Sigma; finally, 3 cases (iritis, neuritis retrobulbaris and hæmorrhage) gave a slight positive result only in the M. test.

The material of non-syphilitic persons, that is, of persons where syphilis can be excluded with the greatest possible security on clinical and anamnestic grounds, was, we need hardly say, rather difficult to select. Certain professions where infections are known to happen frequently, were excluded from the beginning. We strove, moreover, to select, in each case destined to be made use of as a control, the slightest element of suspicion, and to exclude such cases.

We though best to divide our material of controls into two groups. To the first belong, in the first place, completely sound people, or patients suffering from nothing more than a mild local disease, such as a simple eczema or an urethritis anterior. To the second group belong more severe cases : on one side, local diseases with much inflammation; on the other side, diseases of a more general kind, especially tuberculosis, febrile diseases (pneumonia, polyarthritis, etc.), nephritis, tumours; we, moreover, counted here cases of normal pregnancy.

The 340 cases of the first group all reacted negatively with all four methods. Even if a slight indication of positive reaction appeared in one of the four tests, such results did not withstand a repeated investigation.

The reactions appearing in the second group were strong only in quite exceptional cases; they were mostly weakly positive, although they were strong enough to have to be counted as positive results.

The positive cases concern each test in particular in the following distribution : Wassermann, Meinicke and Sachs-Georgi positive : one case of cirrhosis of the liver; W. R. and M. positive : one case of influenza; W. R. and Sigma positive : one nervous disease; W. alone positive : one case of cancer, one of lupus (repeated 3 times), one of polyneuritis and one of polyarthritis; W. and S. G. positive : one pregnancy, an insidious endocarditis and a peptic ulcer; only positive in M. and S. G. : one case of bubo and of peptic ulcer; S. G. and Meinicke positive : one hemiplegia; Sigma alone positive : 5 pregnancies and 3 times peptic ulcer; D. and M. positive : one case of bronchial asthma; lastly M. alone positive : two valvular vices, one case of complaints of rheumatic kind.

Two cases of pneumonia — where alone of all tests the Meinicke gave a reaction of average intensity with the lower dose of serum (2 drops), whereas this test was negative with the higher dose (4 and 8 drops) — have been put with the negative cases, as this sort of result indicates already that the reaction is not specific; we will later on discuss this point.

The consequence of the above-mentioned experiments cannot be due to fortuitous results, as we often had the occasion of repeating the experiment with the same effect. But it was possible to re-examine some cases after some weeks, and all reactions were found negative. If we found very slight indications of a reaction in one of the methods, and if they did not withstand a repeated experimentation, there action was not considered as positive.

Recapitulation of Results.

Our series of investigations shows us the following result :

(1) The W. R., accomplished according to our method, gives, on an average, more often a positive answer than the reactions of precipitation.

(2) The greatest difference between W. R. and the precipitation

tests is found in latent syphilis, but particularly in cases of syphilis under treatment, in tabes, sclerosis and aortitis. Cases of third degree, untreated, are generally positive, with the W. R. as well as with the precipitation tests, with rare exceptions.

(3) The Sigma reaction, of all precipitation tests, shows the most positive results in syphilis, the Meinicke comes second, the Sachs-Georgi test third. But it must be remembered that the M. R. was never performed exclusively with the aid of the original extract sent to us, which proved too weak. We had further to admit as probable that the original Sachs extract that was sent us was weakened through alterations due to despatch.

(4) Although the W. R. is most often positive, one finds not uncommonly cases with a negative W. R. and positive precipitation tests, especially in latent syphilis and in syphilis under treatment. This concerns particularly the Dreyer and Meinicke R.

(5) The ocular cases form a special group, the syphilitic nature of which cannot be proved with absolute certainty. In these cases the precipitation tests showed more often than the W. R. a positive result.

(6) The sera of sound persons, or of patients suffering only from superficial and local diseases, react negatively with all tests. General diseases of various kinds, as well as pregnancy, can give positive results with each one of the tests in about 4.0%. These unspecified reactions are mostly incomplete, nor do they ordinarily show themselves with all four methods.

Part II

General Remarks on the Flocculation Tests.

There is one point common to all precipitation tests that we are now studying : the use of an alcoholic extract of heart as antigen. The principal differences lie : (1) *in the making use of hearts of different animals.* According to our observations, the hearts of calves seem to be the most efficient or all (provided always the same method of preparation is employed), the hearts of oxen would give the weakest antigen, and horses' hearts occupy an intermediate place. (2) *In the different methods of preparing the stock antigen.* Our comparative investigations point to the conclusion that the *Bordet* method, as used by *Dreyer*, gives the strongest extracts. For instance, if we prepare a stock extract of the Meinicke type and another after Bordet-Dreyer, making use the first and second times of the same horse's heart, then diluting both as in the technique of the M. reaction, the extract prepared after the Bordet-Dreyer method will be cloudier and more efficient. (3) A very important differential element is given by the *different ways of diluting the stock extracts.* According to our experience, an extract is, on an average, most efficient when its dispersity is as small as

possible; so that a further diminution of the degree of dispersity would no longer be a colloidal suspension, but would constitute an extract flocculating spontaneously immediately after its preparation. The degree of dispersity of the heart lipoids is dependent—as is the case with other colloids soluble in alcohol and as will be readily understood—on the concentration of saline solution. It depends yet more on the quantitative ratio that exists between alcohol and water at the moment when the colloid state commences. The proportion which allows the least possible degree of dispersity of the colloid in formation, that is, which permits the greatest cloudiness, is a quite precise one for every lipoid soluble in alcohol.

For heart lipoids the optimum will be found in about one part alcohol for half a part water. It appears, therefore, that the Meinicke proceeding for dilution of extracts is a correct one, as it realises this ratio 1 1/2 during the first phase of the dilution. But the degree of cloudiness is yet dependent on the time during which the lipoid undergoes this alcohol-water mixture in the optimal proportions aforesaid. For instance, the Meinicke extract will show after an hour exactly the most adequate cloudiness without flocculation; whereas the calves'-heart extract prepared in the same way would be too cloudy already after one hour. If saline is taken instead of water for this primary dilution, a thick cloudiness will appear much more quickly. A spontaneous flocculation will then appear for a certain concentration of saline in the proportion 1 1/2. One should, therefore, as I could demonstrate in researches made with *Kunewalder*, prepare twice diluted extracts in this way : after having added *saline* for the first time, add the second dose after a much shorter interval; or else, one should choose for the first phase a different proportion of alcohol and water, in the sense that a greater quantity of water be used. The Sachs-Georgi technique of preparing the mixture realises both of these conditions, as in this case we dilute with saline twice in succession, but the first phase is made in the ratio 1/1, and this mixture acts for only a short time on the lipoid. Moreover, a proper extract can also be obtained by fractioning much more; the method that goes farthest in this direction is the drop method, chosen by Dreyer. Its essential particularity is to be seen in the fact that the proportion of alcohol and water that gives the most dense mixture is exceeded only very slowly, in opposition to the rapid dilution accomplished in one turn by other methods. The rapidity with which the drops are added, which gives optimal extracts, must be determined empirically for every extract and for every concentration of saline, as has been done by Dreyer when establishing his technique of dilution of extracts. We will point yet to another fact, that, while preparing the first phase to an optimum, some differences can be found, according to whether water or saline has been added all at once or in drops; whereas, after having attained that critical point, the rapidity of addition plays a relatively insignificant part (Researches upon Mastic, yet unpublished, by *Brandt* and myself).

(4) Another difference lies in the different techniques for *inactivating the sera.* This inactivation suppresses certain circumstances preventing flocculation, as has been shown at first by Sachs and Georgi. Brandt and I could confirm and extend this fact. We found that the Sachs-Georgi technique, that is to say, an inactive method, prevents flocculation of even the strongest syphilitic sera if an active serum is added, even in a relatively small quantity. There is no difference if a normal serum or a syphilitic one is employed in this case.

We could convince ourselves also that this sort of defence against flocculation is not at all identical with the protection conferred by a serum to a colloid against other causes of flocculation, and therefore must not be identified with the protection which can be conferred by any serum added to an extract of lipoids against flocculation by salts (let us notice, by the way, that syphilitic sera also give this protection against spontaneous flocculation, but this can be verified only in certain quantitative conditions; whereas, in experiments conducted under ordinary circumstances the defence against flocculation is overwhelmed by the specific tendency of syphilitic sera towards flocculation). The difference between these two kinds of protection is given by the following characters : certain influences annihilate the protection conferred by active sera against specific flocculation, whereas they leave unchanged the protection against the flocculation of the extract. An example of such influences is given by inactivation or by employing 2% saline solution instead of physiological solution.

The different authors have reckoned with these facts while establishing their methods.

Sachs-Georgi and Dreyer, for instance, have worked with inactivated serum and physiological saline solution, whereas the M. D. can be accomplished with active serum with the same effect, as the saline solution is employed at 2%. The duration of the inactivity also plays a part. It is easy to convince oneself of this in accomplishing the Wassermann test with active and inactive serum. Under certain quantitative circumstances, and in opposition to the views exposed up to now, the technique of using inactive serum gives stronger results than the technique of employing inactive serum; in this case an important part is played by the duration of the inactivation.

(5) The addition of cholesterol to extracts, as recommended by Sachs and adopted in Dreyer's method, has, it is true, the effect of accentuating the reaction, but seems to involve a tendency towards unspecific positive results. It is not yet clear how cholesterol can act in this way. It should be examined more in detail, the extent to which a stronger saline concentration, as employed in the Meinicke technique, can act in the same way as adding cholesterol.

(6) An important factor in obtaining an optimal result lies in the quantitative proportions of serum and antigen. More detailed investigations would be necessary to elucidate this

question, but, before proceeding to an alteration of the quantitative technique, it is necessary to make experiments on a large series of controls, as the specificity of the method would suffer from a too-hasty change. Meinicke has recommended, as a complement to his original method, to enforce the results of the tests by varying the quantity of antigen. We prefer, for technical reasons, to vary the quantity of serum, and convinced ourselves that a double quantity of serum (*i.e.*, 0,4-8 drops instead of 0.2-4 drops) in most cases effectively enforces the positive results; it is even not infrequent that only this dose gives a positive result in syphilitic sera. As a consequence we used also this double quantity of serum in all cases of our series of comparison. On the other hand, we also diminished the serum dose to half its quantity (0.1-2 drops); this corresponds to an experiment with a double dose of antigen. Here also syphilitic cases will exceptionally show an enforcement of results, but this will mostly happen with cases reacting already strongly with a normal dose of serum (0.2-4 g). Only rarely could we find a case of syphilis reacting negatively or weakly with the simple dose that reacted positively with half the dose. On the contrary, it was not rare to find a reaction of this type in diseases not due to syphilis, and therefore it is not allowed to consider as characteristic of syphilis, a positive reaction that only happens with an enforced dose of antigen (or respectively a diminished dose of serum).

For the Sigma reaction the most important doses of serum to be considered are 10, 5, 2 and 1 drops. It will be seen that the quantitative appreciation is foreseen on the same lines as in the Meinicke test. If the dose of serum is augmented to 20 drops, a more concentrated antigen must also be employed. It is only in this way that really strong positive results can be obtained with such high quantities of serum. It is true that with this technique the danger of unspecific results increases. On the other hand it is only of small practical advantage, as admitted by Dreyer himself, to make use of strongly diluted doses of serum. Our experience tells us that this proceeding is not worth being taken into consideration for the reason that sometimes non-syphilitic cases will give weak positive results with such small doses of serum (that is, with relatively strong quantities of antigen). We only had one antigen at our disposal, and therefore we cannot give an opinion on the question if different extracts, applied to the testing of an only case, could be brought to an absolutely equal result, the results being rendered comparable with the aid of the suspension factor.

(7) The original precepts given by the authors concerning the duration of the incubation and the temperature of the room were later on partially corrected. This change in the technique of estimation is probably founded on reasons of principle. It must be borne in mind that with the flocculation tests, as well as with the W. R., even non-syphilitic affections can give positive answers in a slight degree, and these reactions can, up to now, not be discriminated from weak reactions due to true syphilis. The desire

to render a test absolutely specific, above all other aims, at first brought authors to establish as short a duration of the reaction as possible. But this involved a too-small margin for positive reactions in flocculation tests as compared with the W. R. This defect was corrected by extending the duration of reactions, but it must always be borne in mind that weak reactions are only to be taken into consideration in case of absolutely certain syphilis.

Final Considerations.

Our investigations have shown us that the best method proposed up to now is the Wassermann reaction. If the necessary exactness of fulfilment and conscientious estimation of results are realised, it will give, on an average, more valuable answers than the flocculation tests drawn into comparison, provided that sufficiently numerous control cases be disposed of. With an equal specificity, the W. R. will more often be positive, especially in cases of latent syphilis, and still more in cases of syphilis under treatment. A second advantage, not to be disregarded, is that the time necessary for accomplishing the test is much shorter, so that there is also a possibility of repeating the test at a time when the serum of the patient has not been altered. It is true that every W. R. makes it necessary to proceed to numerous preparatory and parallel experiments, because of the necessity of selecting the best possible reagents. As a consequence, it requires a numerous and well-trained staff. For this reason, and because of the selection of numerous normal and syphilitic controls, the W. R. method is recommended only for larger institutes, a near contact with a hospital being a *conditio sine qua non*. The cost of examining a larger number of cases is not much higher than that of a single case, so that, from the economical point of view, it appears commendable to proceed to a certain centralisation of the researches in appropriate institutes of the first importance.

As to the technique itself, we come to the conclusion that *attention must be directed, first of all, to an antigen of optimal efficiency*. A judicious selection and testing of hæmolytic factors—and this especially concerns the complement—a correct preparatory treatment of the sera, the establishment of all necessary controls, a most exact dosing and a correct reading of the results must not be neglected. We are of opinion that this important point (antigen of optimal efficiency) has not been sufficiently considered for the W. R. if we bear in mind the most exact preparation of the extracts for the flocculation tests; we believe that the results of the W. R. could be brought to a much higher degree of efficiency if investigations be undertaken with the view to determining the best way of preparing the antigen. Other searchers should try to elucidate the reasons of positive results in non-syphilitic sera. It would seem to us that other causes than those usually accepted, such as imperfect complement, auto-inhibition, a too-strong action of the alcohol, produce a lack of hemolyse, and

these other factors are not completely identical with those that produce the positive W. R. in cases of syphilis.

Actually we are obliged to count on a certain percentage of unspecific reactions. I believe it would be a mistake to avoid such reactions at any cost (even when a rare exception occurs in severe general infections) by rendering the test less delicate. For this aim could only be realised, if at all possible, by a too-considerable diminution of the margin of reactiveness for syphilitic cases, and this would suppress one of the greatest advantages of the method. The very rare unspecific results in severe general diseases of the non-syphilitic kind will hardly ever do harm, as we can vouch from our practical experience. It will be sufficient to bear this possibility in mind.

One example will suffice. Let us remember the great difference existing between an abortive cure during the true primary phase, but at a time when W. R. is already positive. Considerable harm can be done in that case by an apparently negative result.

In what concerns the flocculation tests in general, the lesser delicacy must first of all be noted as a disadvantage as compared with the W. R.; the sometimes difficult reading and, lastly, the long duration of the test are further disadvantages. As an advantage we have the easier accomplishment and a considerable reduction of expense. It is true that here also the simultaneous testing of numerous and sure control cases must be insisted on, because flocculation tests, for reasons unknown up to now, can give on certain days less valuable results, whether because they are too weak or because they are not sufficiently specific. Consequently the flocculation tests also cannot be accomplished alone; they, too, necessitate an intimate contact with a hospital service. But a smaller staff can do the work, and its instruction is simpler and can be done in less time; this, as well as the absence of costly complements, renders the flocculation tests remarkably cheaper than the W. R.

The Dreyer method proved the best of the three in our comparative investigations. Excepting some positive reactions in cases of pregnancy without syphilis (the cause and constancy of the latter results should be investigated), it is as specific as the W. R. itself and at least as good as the other flocculation tests.

It should be possible to simplify the technical execution by trained hands, so that this test would hardly be more difficult than any of the other flocculation tests and certainly much simpler than the W. R.

We will not examine for the moment the question as to whether any one of the precipitation tests could be perfected so far as to reveal surely all those cases of syphilis that now the W. R. alone can detect. We would be tempted to say that the technique of the complement-binding reaction constitutes the more delicate reagent, so that it can often reveal relations between serum and antigen that will never be accessible, with any certainty, to flocculation tests. Nowadays we certainly must make use of the W. R. as well as of the flocculation tests if we aim at a sure

serodiagnosis in any dubious case, and we are brought to the following conclusion by our investigations : that we are not entitled, for the moment, to renounce any of the flocculation tests, for, although the Dreyer method gives the most numerous positive answers, there occur also with the Meinicke as well as with the Sachs-Georgi, single positive reactions, and these cannot be missed in the study of the question if latent syphilis exists in a given case.

A COMPARATIVE EXAMINATION OF METHODS OF SERODIAGNOSIS OF SYPHILIS : BORDET-WASSERMANN, MEINICKE (D. M.), SACHS-GEORGI AND SIGMA REACTION

Report by Dr. E. Renaux, Pasteur Institute, Brussels

The present note is a supplement to the preliminary report sent a few months ago, copies of which have already been forwarded to the institutes participating in this enquiry. In its broad outline, it does not differ from the previous report as regards the conclusions relating to the Sachs-Georgi and Meinicke reactions. On the other hand, those which relate to the Sigma reaction are partially modified. At the instance of Professor Dreyer, we have been led to alter the types of tubes which we originally used and the diameter of which did not correspond to that indicated by the author; the inner diameter of our tubes was from 7.8 to 8.2 mm., instead of Dreyer's measurement of 5.5 to 6.2 mm. (1).

We have been able to ascertain that the use of tubes of an inner diameter of 6 mm. considerably increases the percentage of positive results obtained by this method and makes the reading of the results much easier. In view of this alteration, we have thought it desirable to provide a special table of the experiments which we carried out under these conditions.

We maintain our general remarks on the Meinicke and Sachs-Georgi reactions and the difficulty of the reading of the results. The miscalculation which occurred with the Sigma reaction induced us to consider the importance in these two methods also of the diameter of the tubes used. This investigation is now in progress.

One remark must be made on the classification of cases : Until quite recently we had not considered the term "*latent* syphilis", and had grouped under the heading "*treated syphilis*" all cases which had undergone treatment sometime or other. We considered it difficult to omit classifying as "syphilis under treatment" all cases in which the patient, after undergoing several periods of treatment, is resting for one, two or three months before receiving another series of therapeutic injections. We have therefore been unable, in a certain number of cases, to discern with certainty those under present and immediate treatment from those who had been without treatment for one or two months. Cases which were

(1) As we were unable quickly to procure on the spot, tubes of an average diameter of 6 mm., we did not think it necessary to delay our experiments, for a reason the full importance of which we did not then realise.

uncertain in this respect have been grouped with the cases of *"latent syphilis"*. The figures for this category may have been falsified on this account to the extent of from one to two per cent.

Table I.

General summary of positive results reached in the various categories by the four methods.

	No. of Cases	B. W.	M.	S. G.	Sigma-reaction
S. I not treated	16	13	13	13	14
S. II »	69	68	63	67	68
S. III »	50	48	38	45	45
S. latent	635	243	184	234	256
Tabes not treated	15	10	5	6	8
D. P. not treated	9	9	5	6	7
S. under treatment	70	22	22	22	27
Total S.	864	413	330	393	425
Control cases	541	0	5	28	10

Table II.

Note. — The cases mentioned in Table II are also included in Table I, and must therefore not be added in order to arrive at the total. This table merely shows the difference produced in the results of the Sigma-reaction by the use of more adequate material.

	No. of Cases	B. W.	M.	S. G.	Sigma-reaction
S. I not treated	7	6	6	6	7
S. II »	13	13	12	13	13
S. III »	16	16	10	15	16
S. latent not treated	138	61	42	61	91
Tabes not treated	5	4	2	3	5
D. P. not treated	0				
S. under treatment	10	5	5	5	9
Total S.	189	105	77	103	141
Control cases	100	0	2	7	2

Summary Description of the Control Cases giving a Positive Reaction

I. *Bordet-Wassermann.*

We found no non-syphilitic cases giving a higher degree than that indicated here by the words "long delay in hæmolysis", that is, complete hæmolysis in an average of 60 minutes instead of 30 minutes.

II. *Meinicke D. M.*

111. Aortic dilatation : weak positive (see also S. G.).
112. Ganglial tuberculosis : weak positive (see also S. G.).

1010. Pleural effusion : probably tuberculosis; weak positive.
1201. Nephritis : positive (see also S. G.).
1202. Woman in good health who had had, some years ago, three still-born full-time children in succession; positive. We were able to test again numbers 1010 and 1202, with identical results.

III. *Sachs-Georgi.*

32. Patient in good health; weak positive (see also Sigma reaction).
111. Aortic dilatation : weak positive (see also Meinicke).
112. Ganglial tuberculosis : weak positive (see also Meinicke).
135. Appendicitis : positive.
379. Pulmonary tuberculosis : positive.
383. Pulmonary tuberculosis : positive.
545. Anæmia and adenitis tuberculosis : positive.
555. Chlorosis : positive.
568. Pulmonary tuberculosis : weak positive.
570. Pulmonary tuberculosis : weak positive.
644. Tuberculosis bronchitis : positive.
683. Patient in good health : weak positive with one antigen, negative with the other.
694. Planter perforation : positive with one antigen, weak positive with the other.
895. Hemiplegia : weak positive with one antigen, negative with the other.
1008. Hemiplegia : weak positive.
1043. Cerebral tumour : positive.
1086. Tuberculous osteitis with adenitis : weak positive.
1160. Rhinopharyngitis : positive (see also Sigma reaction).
1165. Patient in good health : weak positive.
1169. Optical neuritis : weak positive.
1174. Patient in good health : weak positive.
1201. Nephritis : weak positive (see also Meinicke).
1211. Acute articular rheumatism : positive.
1214. Gastric ulcer : positive.

It should be noted that of these 24 positive reactions six were carried out twice with the same result (32 and 1165, 379 and 571, 383 and 568, 545 and 1086, 683 and 1174, 895 and 1008). The real number is thus reduced to 18. Thirteen reactions are weak positive. Strictly speaking, cases in which there is no record of syphilis might be disregarded. Eight cases occurred in tuberculous patients. They can actually be reduced to five, as three of them were examined twice with similar results (379 and 571, 383 and 568, 545 and 1086).

Sigma Reaction.

32. Patient in good health : weak positive : 1.1 Units (see also S. G.).

69. Atrophy of the optic nerve : weak positive : 1.1 Units.
80. Post-partum : weak positive : 1.3 Units.
102. Sclerosis : *en plaques* positive : 2.2 Units.
196. Cerebral tumour : weak positive : 1.1 Units.
785. Glycosuria : weak positive : 1.1 Units.
1069. Varicose ulcer : weak positive : 1.1 Units.
1101. Chronic rheumatism : weak positive : 1.3 Units.
1160. Rhinopharyngitis : weak positive : 1.2 Units (see also S. G.).
1278. Psoriasis : weak positive : 0.89 Units.

Number 32, when re-examined as No. 1165, gave a negative result. The other samples could not be re-examined. All these positive results, except No. 102, gave a number of units less than 1.4. They might therefore be disregarded in the absence of any record or any clinical symptom of syphilis. Unfortunately, we were unable to obtain another sample from No. 102, who was a very difficult patient and would not permit any further treatment

(a) *The diagnostic value of methods examined.* — Even if nothing but clear flocculation be considered, the Meinicke and Sachs-Georgi tests have given indisputably positive results in which no symptom or record of syphilis could be found. The Sigma reaction also offers a case of this kind. But beside the "clear flocculations" there is also a series of cases in which flocculation is more or less intense, and is described as "slight, very slight, or traces."

It is important to be able to form, by means of titration, a fairly precise idea of the intensity of the reaction in these circumstances; it is in this respect that Dreyer's method presents advantages, as the expression in units gives a basis for the differenciation of highly specific results from those which have not that character or which are doubtful. This basis, though empirical, admits of a more precise comparison between the results reached in the various laboratories.

But the diagnostic value of methods depends also upon the significance of the negative reaction; it seems certain that with the strict technique of the Sigma reaction, a greater number of positive results is obtained, particularly in cases of latent syphilis. We have not been able to apply this technique widely enough to express a final opinion on it. The Meinicke reaction has always seemed to us definitely inferior to the others.

(b) *Complexity of technique.* — As we said in our preliminary report, the difficulty of flocculation reactions consists in the fact that their readings are sometimes difficult. Due weight must be given to the less definite tests which sometimes make the diagnosis uncertain. It is to be noted, for instance, that the Sigma reaction, even if carried out with a standard flocculation, often still leaves us in doubt as to the degree of flocculation observed, and this affects the number of units attributed to a given serum. In order that the results of different experiments may be comparable, it is desirable, though not strictly necessary, that

the reading should be taken by the same operator, working under the same conditions.

In general, the flocculation reactions have the advantage of not needing, as does the Bordet-Wassermann reaction, various substances each of which requires titration. However, the Meinicke antigen and the Sachs-Georgi antigen when used with NaCl at a strength of 15 per 1,000 have sometimes an unfortunate tendency to auto-flocculation.

(c) *Time required.* — The Bordet-Wassermann reaction gives the quickest results, as the entire process may be carried out within 8 hours. In practice, however, most of the laboratories in Belgium carry out this reaction two or three times a week, so that they have always from 60 to 80 sera to examine at one time.

(d) *Cost of analysis.* — A little higher for the Bordet-Wassermann reaction, owing to the necessity of maintaining animals for experiment.

(e) *Facility of performance and accuracy.* — In our opinion the observation of the Bordet-Wassermann reaction is still the most accurate and the most obvious. The Sigma reaction is more difficult to read and needs more experience than the Meinicke and the Sachs-Georgi; it is, however, fairly convenient in the long run when it has been sufficiently practised.

(f) *Percentage of dubious results.* — This percentage is, on the whole, fairly small. With the Bordet-Wassermann reaction there are cases in which the serum is entirely anti-complementary. This seldom happens, and very often, by placing the tubes in the water-bath at 40 to 45°, we obtain a sufficiently well-marked hæmolysis to enable us to give a fairly precise answer; small differences, of course (delay in hæmolysis and differences of a degree), are not shown by this method. Only two cases appeared quite impossible to determine.

With the Meinicke reaction we have several times found flocculation in all the tubes, which obliged us to recommence the tests. In general, however, there is for flocculation reactions a perceptible minimum degree which may sometimes give one pause, particularly where more or less septic sera are concerned.

(g) *Quantitative titration.* — We have been carrying out quantitative titration for the Bordet-Wassermann reaction for 12 years, using a technique which is identical in principle to that of the Sigma reaction : gradually decreasing doses of serum and a fixed dose of antigen. On the whole, the results of the titrations of the Sigma reaction fit in well with the former, though there are sometimes marked discrepancies in which it is impossible to decide which indication is the more accurate. However, when we followed up with successive examinations of the patients under treatment, we obtained very similar curves, except in two cases, in which, with our original test-tube material, we obtained 1.3 and 1.8 units respectively, with weak positive (±) Bordet-

Wassermann reactions; a final test recently carried out gave us a negative Bordet-Wassermann reaction when the Sigma reaction carried out with the new material gave 3.2 and 2.9 units.

The titration experiments which were carried out for the Sachs-Georgi and Meinicke reactions gave us some incompatible results. In some cases we found perfect regularity and agreement, but in others (26%) it was almost impossible to form an estimate.

THE 1,000 FIRST COMPARATIVE SERODIAGNOSTIC EXPERIMENTS WITH THE WASSERMANN REACTION AND THE FLOCCULATION METHODS OF SACHS-GEORGI, MEINICKE (THIRD MODIFICATION) AND DREYER-WARD (SIGMA REACTION)

Report by Professor H. Sachs, Heidelberg

In the following memorandum, I venture to report on the results of the first 1,000 serum tests which were carried out, according to the decisions of the Conference held in London on December 12th and 14th, 1921, in co-operation with Dr. Felix Georgi, Miss Helene Rosenstein and Miss Lily Abraham.

The Sigma reaction of Dreyer-Ward could not be immediately tested because the necessary reagents and apparatus were received somewhat late from London and Oxford. I have therefore ventured to append a report (see Annex) on 1,025 further cases, the investigation of which had been begun previously and only concern the Wassermann reaction and the flocculation methods of Sachs-Georgi and Meinicke. The results appear, generally speaking, to correspond with those recorded in the main report.

Cerebro-spinal fluids were not at first taken into consideration, but they will in future be subjected to parallel tests in as large a number as possible.

As regards the method, the procedure agreed on in London was scrupulously observed. When using the Sigma reaction, it was not found possible during the first part of the experiments to take readings by means of the lighting apparatus (agglutinoscope) suggested by Dreyer-Ward, because the apparatus had not been received in time from Oxford. However, the previous and subsequent results were so largely in agreement that all the results can be taken into consideration. The use of the agglutinoscope constructed by Dreyer and Ward undoubtedly makes the readings much easier. In the remainder, the rules indicated by these authors were scrupulously observed.

The Wassermann reaction was carried out according to the German Government regulations. In accordance with these instructions, the results had fairly often to be registered as "suspicious" (?) that is, when a complete or almost complete inhibition of hæmolysis was to be observed only with the latter part of extracts used.

Survey of the Results obtained.

A summary of all the results obtained is given below in tabular form. The following abbreviations have been used :

WaR = Wassermann reaction.
SGR = Sachs-Georgi reaction.
MR = Meinicke reaction (third modification).
Sigma R = Dreyer-Ward reaction.

Reactions obtained with :

WaR	SGR	MR	Sigma R	Number of sera
+	+	+	+	158
−	−	−	−	757
−	+	+	−	5
+	+	−	+	11
+	+	−	−	3
+	−	−	−	6
?	+	+	+	9
?	+	−	+	12
?	+	−	−	6
?	−	+	+	1
?	−	−	+	3
?	−	−	−	9
−	+	+	+	3
−	−	+	+	1
−	+	−	+	1
−	−	+	−	1
−	+	−	−	2
−	−	−	+	12
		Total		1,000

It will be seen from this summary that 915 sera (91.5%) showed complete concordance of results by all four methods. If the results of the methods taken in groups of three are compared, the numbers in which uniform results were obtained are as follows :

Reactions obtained :

With	Concordant results		Total
	Positive	Negative	
WaR-SGR-MR . . .	163	769	932 — 93.2%
WaR-SGR-Sigma R .	169	758	927 — 92.7%
WaR-MR-Sigma R. .	158	759	917 — 91.7%
SGR-MR Sigma R . .	170	772	942 — 94.2%

It will be seen that concordant results are most numerous when the Sachs-Georgi, Meinicke and Sigma reactions are compared. It must, however, be recognised that, considering the relatively small quantities of material employed, too much importance should not be attached to the divergencies.

Finally, the following summary shows the results of a comparison of the methods by pairs.

Reactions obtained :

With	Concordant results		Total
	Positive	Negative	
WaR-SGR	177	731	948 — 94.8%
WaR-MR	163	770	933 — 93.3%
WaR-Sigma R. . . .	169	760	929 — 92.9%
SGR-MR	175	787	962 — 96.2%
SGR-Sigma R. . . .	194	773	967 — 96.7%
MR-Sigma R	172	783	955 — 95.5%

As regards the diseases to which the 85 sera giving different answers refer, the great majority of these were of syphilitic or metasyphilitic nature. It is true that in some cases reliable indications could not be obtained for a positive syphilitic clinical history. It would not appear admissible, of course, to exclude a previous syphilitic infection in any individual case. A case to which attention should be drawn is one of multiple sclerosis in which the Wassermann reaction was dubious and all three flocculation reactions were negative; and also a case of polyarthritis which gave a positive Wassermann reaction and a negative result for the flocculation reactions. There was also a case of malaria which only gave a positive reaction with the Wassermann and Sachs-Georgi tests. As regards cases in which there was a negative Wassermann reaction and positive results with one or more methods of flocculation, they concern—so far as there were no indications of a syphilitic infection — one case of jaundice which only reacted positively with the Meinicke reaction, and three cases of tuberculosis, sciatica, and one disease which could not be diagnosed, in all of which the Sigma reaction alone was positive.

In regard to the circumstance that in a considerable number of sera the Sigma reaction alone proved positive, it must be borne in mind that we were mostly dealing with very weak positive reactions which only appeared in the first tubes of the Dreyer-Ward experimental apparatus, so that it should perhaps be considered whether such weak reactions, which only appear with the largest quantities of serum used in the Sigma reaction, ought still to be regarded as positive. If we consider the greater quantities of serum used, the conditions of the Sigma reaction would be effectively near to the point where the test approaches its most near specific character.

Comparative examination of the result after seven hours, as required by the original Dreyer-Ward prescription, and after from twenty to twenty-two hours, in accordance with the more recent directions given by Professor Dreyer, tends to confirm this opinion. In the first 744 experiments with which this report deals, the readings taken after seven hours were used as a basis, and in the subsequent 256 experiments they were taken after the tubes had been in the water-bath for a period of from twenty to twenty-two hours.

It thus appeared that the method is giving increasingly good

results from the point of view of delicacy, but that, with regard to the first two test-tubes containing a larger volume of serum, there is a greater liability to non-specific reactions. This is in complete agreement with the results obtained by comparing the earlier and the later readings. It was seen previously that in the Sigma reaction, when readings were taken after twenty or twenty-four hours, non-specific reactions appeared to occur frequently in the first test-tubes. Later readings may nevertheless prove to be indicated since, so far as previous observations permit any conclusion to be drawn, positive reactions continue to be produced and progressively appear in test-tubes containing smaller quantities of serum. On the other hand, in the case of positive sera, inhibition of the reaction could occasionally be observed in the first tubes after 20 hours, as recorded already by Dreyer-Ward. My researches serve to show that flocci occurring only in the first two test-tubes will have to be disregarded when later readings are taken. For this reason, when dealing with the Sigma reaction, I have also considered as negative reactions flocculations which were to be observed only in the first two test-tubes at the time of the later reading.

This point of view has been adhered to in the following table, which shows the results in accordance with the plan accepted by the London Conference. Under the heading of the Wassermann reaction, such sera are marked with "?", which give positive reactions of greater or less strength, only with a minority of the extracts employed, in accordance with the German Government regulations for carrying out the Wassermann reaction.

Result of Serum Tests.

	No. of cases	Wa R			M R		S. G R.		Sigma R.	
		+	?	—	+	—	+	—	+	—
Syphilis I. Not treated.	21	7	2	12	7	14	7	14	9	12
Syphilis II. Not treated.	57	51	2	4	52	5	52	5	50	7
Syphilis III. Not treated (1). . . .	70	44	3	23	41	29	46	24	45	25
Latent syphilis. . . .	145	40	13	92	39	106	53	92	52	93
Tabes. Not treated.	10	1	1	8	2	8	2	8	2	8
Paralysis. Not treated	7	6	1	0	4	3	7	0	7	0
Syphilis. Under treatment.	94	32	17	45	32	62	42	52	43	51
Syphilis. Total number of cases . . .	404	181	39	184	177	227	209	195	208	196
Control cases (2). . .	596	2	1	593	1	595	1	595	3	593

(1) The following are included under the heading of "Syphilis III":

5 sera from Syphilis cerebri
6 from Keratitis parenchymatosa
3 from Syphilis congenit
6 from Iritis.

(2) The following diagnoses occur in the cases giving apparently a non-specific reaction:

1. Polyarthritis (Wa R + else —)
2. Multiple Sklerosis (Wa R ? else —)
3. Malaria (Wa R & Sachs-Georgi + else —)
4. Ikterus gravis (Meinicke + else —)
5. Sciatica (Sigma +, else —)
6. Tuberculosis (Sigma +, else —)
7. Diagnosis could not be obtained (Sigma +, else —).

In the cases 5 and 6, weak Sigma reactions were obtained in the first three and four tubes respectively.

In this table it should be noted that the cases indicated as doubtful as regards the Wassermann reaction should probably mostly be regarded as belonging to the positive column. This fact accounts for the comparatively small number of absolutely certain positive results. The concordance on the whole is fairly close, though the Meinicke reaction appears to be somewhat inferior to the other tests from the point of view of delicacy.

A few remarks have still to be made in connection with the 757 cases which gave uniform negative reactions with all methods. The cases consist of the most various types of diseases and include a fairly large number of sero-negative syphilis cases. It will probably be sufficient for the moment to point out that under this heading there are 20 cases of tuberculosis, 3 of ulcus molle, 14 of gonorrhœa and 30 of tumours, and that therefore we are dealing with diseases which, as experience shows, tend most easily to show a non-specific serum modification. It follows that in the

material hitherto at our disposal there is no evidence of the frequent occurrence of uncharacteristic results in definite forms of disease. The relatively abundant material which is here submitted with regard to control cases may accordingly prove of some value for the comprehension of specificity itself.

As regards the special points in connection with which the report should, in accordance with the London Resolutions, supply information, the following questions are to be dealt with :

(*a*) *Diagnostic value.* — The investigations which have so far been carried out scarcely permit of any final opinion being emitted on the question of diagnostic value. Two important points have to be considered : delicacy of the test and characteristic behaviour in the case of syphilis.

The Meinicke test appears to be the least satisfactory from the point of view of delicacy. This is perhaps due to the fact that in the Meinicke reaction the dilutions of extracts do not always turn out to be absolutely uniform, or we get strong positive reactions—and then slight flocculations may frequently be observed in negative sera or in extract controls—or else we find the diluted extracts homogeneous in the controls and in negative sera, then even sera which are slightly positive give a negative or only a very weak reaction.

As regards delicacy, the Sigma reaction and the Sachs-Georgi reaction have hitherto proved practically equal. It is true that in the case of early readings (first 744 experiments) the reactions in the largest volumes of serum, too, were taken into consideration, and that, as stated before, *may perhaps account for uncharacteristic reactions.*

If the strongly marked positive reactions alone are taken into account, the Wassermann test, as regards delicacy, occupies a position between the Meinicke reaction and the other two methods, but slightly superior to the latter even when the "doubtful" cases are accepted as being positive.

As regards uncharacteristic reactions, none of the methods have as yet given a greater number of positive results in any definite non-syphilitic type of disease. Experience in connection with the Sigma reaction, however, seems to indicate that the conditions under which the experiments are carried out may be too rigid, inasmuch as the largest volumes of serum are taken into account, especially when taking later readings. In the Sigma reaction, as in the Sachs-Georgi reaction, the principle consists in the cholesterination of extract, with the only difference that the raw extract is prepared differently. It is conceivable, perhaps, that the divergences are only due to the difference in the cholesteral content and in the volumes of serum.

The diagnostic value of flocculation tests may be considered to lie in the fact that flocculation sometimes gives a positive result in cases where the Wassermann test gives negative or only doubtful results. On the other hand, cases which give positive results in the Wassermann test may react negatively under flocculation

methods, so that in order to reduce to the minimum the margin of error where syphilitic sera are concerned the best method would appear to be a combination of the Wassermann reaction and of the flocculation tests. It is beyond doubt, however, that high diagnostic value can be claimed for the flocculation tests alone as well as for the Wassermann reaction alone, but the Meinicke reaction possesses a lesser delicacy.

(*b*) *Complexity of technique.* — Flocculation tests appear to be technically simpler than the Wassermann reaction, for the simple reason that the former require only two constituents. The drop-by-drop method used in the "Sigma" test undoubtedly presents difficulties; for more practised investigators, however, its only drawback is the fact that it requires more time.

In order to avoid error due to auto-flocculation, it is advisable in *every* flocculation test to set up, in principle, serum controls (without addition of extract) as well as extract controls (without addition of serum).

(*c*) *Time required.* — As was said above, more time is required for measurement drop by drop, as in the "Sigma" test, than when graduated pipettes are used. Consequently the Sachs-Georgi and Meinicke tests can be carried out more rapidly, even if the prescribed quantitative formula for the "Sigma" test is taken into account. The Wassermann reaction requires the longest time of all. On the other hand, the reading of the results given by the Wassermann test requires least time, considering the prescriptions hitherto in use for flocculation tests. It may be possible, however, to find a means of shortening the time required for reading results in flocculation methods. In the Sachs-Georgi test the results of the vast majority of cases can be estimated within from 3 to 4 hours. The only danger consists in the occasional occurrence, after short observation, of unspecific reactions which subsequently disappear. Experimental research would therefore have to be made in order to ascertain whether, by using an adequate mode of procedure, it would be possible, under flocculation methods, to make results discernible at an earlier stage while avoiding the risk of obtaining uncharacteristic results. The centrifugal method and the decantation test ("Ueberschichtungsprobe") are also at present being employed for investigations in this connection.

(*d*) *Cost.* — The Wassermann reaction involves greater expenditure than the flocculation test, since it requires the use of guinea-pigs, sheep's blood and haemolytic amboceptors.

(*e*) *Reading.* — The reading of results in flocculation tests generally requires a more practised eye than in the Wassermann reaction, but all difficulties disappear after sufficient experience has been gained. It is advisable that the result should only be classified as positive when the flocci are quite definitely distinguishable. Using the state of turbidness as a *criterium* has hitherto presented no advantages, even in early readings.

(*f*) *Percentage of doubtful cases.* — Doubtful cases may occur with the Wassermann test by reason of an auto-inhibition caused by the serum or with flocculation methods by reason of spontaneous flocculation of the serum. We have not met with either of these irregularities in the experiments reported here, and according to our general experience they are of the utmost rarity. It is, of course, also desirable, as we have already pointed out, to test the sera for spontaneous flocculation when flocculation methods are employed. For this reason, a control serum was set up also for the Sigma test, and 10 drops of serum were mixed with an alcohol dilution corresponding with the extract dilution.

Moreover, doubtful results scarcely occur in flocculation tests except when the control extracts disclose spontaneous flocculation. In the Sigma and Sachs-Georgi reactions such spontaneous flocculation in the control extracts was disregarded; in the Meinicke test such spontaneous flocculation did occasionally appear. The result was, however, classified as negative except when the flocculation was noticeably more pronounced than in the control extracts.

As regards the Wassermann test, the sera which gave complete inhibition of haemolysis only with the minority of the extracts used were classified as doubtful (?), as has already been observed.

(*g*) *Advantages of quantitative titration.* — It is difficult to give any opinion on this question at the present juncture. In the Sigma test it is necessary to work with decreasing quantities of serum inasmuch as a negative reaction is sometimes obtained when larger volumes of serum are used and the optimum point is situated near to the smaller quantities of serum. It would, however, appear possible that when later readings are taken, as now prescribed, a smaller volume of serum may be insufficient, which would, at the same time, represent a saving of material.

Quantitative titration can, of course, also be carried out by means of the Wassermann reaction and the Sachs-Georgi and Meinicke flocculation tests. Up to now, however, experience has by no means proved that quantitative titration offers such advantages as to compensate for the increased labour which it entails.

Conclusion

The experiments under consideration indicate that the flocculation tests show far-reaching parallelism both with the Wassermann test and with each other. It has so far not been possible to obtain complete concordance by the comparative testing of any two methods. There would appear to be syphilitic sera which show a more pronounced reaction either with the Wassermann reaction or with the flocculation test, or only with the one or the other of the flocculation methods. These divergences chiefly occur in cases of syphilis which have been treated and during the period of latency. The various methods are therefore complementary to each other, and in that sense the flocculation tests can already

be regarded at least as valuable auxiliary proceedings for the serodiagnosis of syphilis.

With regard to the value of the various flocculation reactions, the third modification of Meinicke is inferior in delicacy both to the Sachs-Georgi test and to the Sigma test according to the experiments which are reported here. Whether in this case systematic inferiority exists or not will only be ascertained by more extensive series of experiments.

From the technical point of view and that of cost, the flocculation method is more advantageous than that of complement-fixation, and in carrying out further experiments, it will have to be regarded as advisable to determine the most suitable methods, so that diagnostic value as regards delicacy procedure, as well as strict specificity, will unite with simplicity of technique and method. The method of preparing the extract would appear to be the question of vital importance in flocculation tests as in the Wassermann reaction. With reliable extracts, flocculation may, even at this stage, be considered an absolutely satisfactory method of serodiagnosis of syphilis. In order to provide a margin of safety against experimental errors, it is advisable to use at least two different extracts. By setting up parallel experiments with the Wassermann reaction and the flocculation test, the reaction is tested on a broader basis and the results obtained are more reliable.

Annex

Report on 1,025 Comparative Serodiagnostic Experiments with the Wassermann Reaction and the Flocculation Reactions of Sachs-Georgi and Meinicke (Third Modification).

By Professor Dr. H. Sachs, Heidelberg.

As previously stated the experiments reported in the present Annex were made before the tests described in the main report. An extract provided by Geheimrat Kolle at Frankfort and prescribed by Meinicke for these experiments was used for Meinicke's third modification.

The following table will show the total results :

The reactions obtained were as follows :

Wa. R.	S. G. R.	M. R.	Number of sera
+	+	+	159
—	—	—	766
±	±	—	19
±	—	—	4
?	±	±	9
?	+	—	15
?	—	—	42
—	—	±	2
—	±	—	7
—	—	+	2
			1,025

According to this table, 925 sera, *i.e.*, 90.25%, showed absolute concordance of results when tested by all three methods. In this table, the concordance between the results obtained by these three methods is somewhat less marked than in the tables shown in the main report, *i.e.*, 91.5%.

If we compare the results obtained by any two methods only, we get the following figures :

Method	Positive	Negative	Total
WaR-SGR.	178	768	946 = 92.29%
WaR-MR	159	773	932 = 90.93%
SGR-MR.	170	812	982 = 95.8%

In this table also the percentage of concordant results varies but little as compared with the figures given in the main report. Differences in the material used for the experiments might be sufficient to account for such divergences.

It must be pointed out that of the 766 cases showing a uniform negative reaction, 31 sera were derived from cases of tuberculosis, 8 from gonorhoea, 3 from ulcus molle and 21 from tumours. It cannot therefore be said that with any of the methods employed a larger number of positive reactions has been observed in definite types of disease.

The vast majority of the cases in which samples of serum gave divergent reactions were cases of syphilitic or metasyphilitic diseases; this applies quite particularly to those sera with which positive reactions were obtained only by the Meinicke method or only by the Wassermann method. It also applies to 6 out of the 7 sera with which a positive reaction was obtained only by the Sachs-Georgi method. One serum included among those with which a positive reaction was obtained only by the Sachs-Georgi method came from a case of ulcus molle. The reaction developed comparatively late and was obtained only with one out of the three cholesterinated extracts used.

In conclusion, it may be said that these earlier experiments gave very much the same results as those discussed in the main report.

SERODIAGNOSIS OF SYPHILIS

PRELIMINARY OBSERVATIONS (FROM THE LABORATORY POINT OF VIEW) BASED ON THE EXPERIENCE OF APPROXIMATELY 800 TESTS

By E. J. Wyler, London.

Introduction.

In accordance with the conditions laid down for this investigation at the International Conference of December 1921 :

1. I have not had access to the clinical or pathological records of the cases.

2. Before commencing the investigation I studied the respective authors' own methods in the respective institutes.

3. The samples of serum tested were known only by numbers.

4. Only those extracts prepared by their respective authors were used.

Methods adopted for rendering the tests as strictly parallel as possible :

(*a*) The specimens of blood in each batch of tests could not, for practical reasons, be all collected on the same day. There is therefore an unavoidable variation of a few days in the ages of the specimens. The specimens were stored in the ice-box.

(*b*) Each batch of sera to be tested was withdrawn from the clot on one and the same day (with the exception of a relatively small number of sera obtained from sources other than the venereal clinic at St. Thomas's Hospital). The sera were at once inactivated and stored in the ice-box and the various tests were all put up on the following day. The dilutions of serum for the Sigma test (for practical reasons and as permitted by the authors) were made on the day of inactivation.

(*c*) All tests were carried out by myself only.

There were 37 test-days.
The tests left no time for a detailed report.
If further details are required than are contained in the following observations, they can be supplied.

I. Complexity of Technique

Sigma Test.

Non-specific flocculation. — With the introduction of incubation periods of 20-22 and 40-44 hours (used in approximately the last 400 tests (1), the occurrence of non-specific flocculation, due apparently to infection, has caused difficulty. This has, as a rule, been easily distinguished from true flocculation and has not greatly interfered with the readings. It would appear to be largely overcome by using, at Dr. H. M. Ward's suggestion, lithium chloride 1%, instead of sodium chloride 0.85%, for dilution of sera and antigen, together with dry sterilisation of syphon apparatus. This was used in 5 batches of tests totalling 101 sera. Dry sterilisation alone did not abolish infection. But it would seem that lithium chloride itself tends to cause, with some sera, a slight non-specific flocculation, and Professor Dreyer has now advised that its use be abandoned.

In the testing of the sera which gave non-specific results (*vide* Colonel Harrison's report), lithium chloride was not used.

Further experiments are now in progress, under the kind advice of Professor Dreyer and Dr. Ward, with the object of determining the cause of the non-specific flocculation with sodium chloride.

Flocculation in extract controls. — On 13 test-days out of a total of 37, flocculation was observed in the Alpha or Beta controls. In eleven of these it was tr— or less and therefore, according to Dr. Ward's advice, negligible.

In two it was tr+ and S in the Alpha controls. These occurred on days when non-specific flocculation (mentioned above) in the test proper was seen and necessitated the cancelling of positive results obtained with the Alpha tube only.

Conclusion. — The technique of this test is simple but laborious, whilst much special apparatus is needed (as described in the author's article—*Lancet*, 1921, I, 956). Since the publication of this article the use of a syringe for the addition of the Beta dilution of antigen to the tubes has been introduced. This is, of course, much less troublesome than adding it with the dropping pipette but necessitates very careful standardisation of the volume of fluid delivered by the syringe against the dropping pipette.

It was early observed that changes of atmospheric temperature which were at all marked adversely affected the accuracy of the syphon apparatus for dilution of antigen, and that it was desirable to check this on each test-day. It has accordingly been my invariable rule to check the apparatus immediately before use.

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(1) Since and including August 1st, 1922.

It may perhaps also be mentioned that an apparatus is needed for maintaining a constant level of water in the bath.

The most laborious parts of the test are undoubtedly the adding of serum and saline to the tubes and the shaking of each individual tube after the addition of the antigen dilutions. It has to be realised that in the performance of only 50 tests (each comprising nine tubes) no less than 450 tubes have to be inverted on the thumb and shaken with due care that no fluid remains on the thumb.

The amount of blood needed to carry out a full test, 4-5 c.c. might in some cases—particularly in young children—create difficulty.

Sachs-Georgi Test.

Flocculation in serum controls. — This, for a time, caused considerable difficulty and on some test-days occurred in about 25% of the tests. It did not appear to bear any definite relation to the age of the serum, and no fallacy was discoverable in the glass-washing routine, which was carried out by a reliable and experienced assistant. Acidified tap-water, plain tap-water and finally distilled water had been used. However, the periodical use of soda in washing glassware, and also the occasional use of bichromate of soda dissolved in sulphuric acid appears to have abolished this trouble. The matter is mentioned here because the glass-washing technique would seem, in flocculation tests, to be of fundamental importance.

It should be added that Professor Sachs informed me in a letter that running water only is ordinarily used for cleaning test-tubes in his laboratory at Heidelberg.

As is well known, spontaneous flocculation is occasionally found in the controls of some sera, and this has necessitated the rejection of a small proportion of tests. Most of these sera were found to have flocculated before dilution, and only very occasionally was it found possible to clear them either by prolonged centrifuging or by filtration through "extra hart" filter-paper of Schleicher and Sehüll (No. 602) as recommended to me by Professor Sachs.

Flocculation of extract dilutions. — The dilution of the extracts was at first carried out strictly according to instructions, an interval of 30 seconds being allowed between the first and second additions of saline. After some weeks, however, it was found that spontaneous flocculation tended to occur either immediately following the second addition or within a minute or two both with Extract II and Extract IV. This flocculation did not occur consistently at first, so that it was possible after perhaps three or four attempts to obtain an unflocculated dilution.

Later it was only possible to obtain unflocculated dilutions by reducing the interval between the two additions of saline to

two or three seconds. The antigenic power of the extract (except on one test-day) did not appear to be affected by this procedure as far as could be judged by comparison with the results obtained in the other tests.

Various attempts were, however, made to overcome this flocculation difficulty, but without success. Saline and distilled water from other laboratories were tried. Special Jena glass and "Duroglass" test-tubes were obtained. A long correspondence on the matter was carried on with Professor Sachs, who kindly sent me some saline dilution prepared in his laboratory and also a further supply of the same batches of extract which, he stated, behaved satisfactorily in his hands. With me, however, flocculation occurred as before. Professor Sachs confirmed the occurrence of spontaneous flocculation in a sample of his undiluted extract which I returned to him.

Many further experiments which cannot be given in detail in this short report were made, but unsuccessfully.

Professor Sachs informed me that slight spontaneous flocculation of the extract dilutions re-dissolves on incubation. I have confirmed this, but in carrying out the test it is not easy to judge the precise degree of flocculation which can be relied upon to re-dissolve.

Conclusion. — The technique of the Sachs-Georgi test is simple to carry out and presents no difficulties other than those referred to above.

No special apparatus is needed with the exception of the agglutinoscope.

Meinicke No. 3 Test.

Flocculation in serum controls. — Serum controls are considered unnecessary by the author of this test. These were nevertheless put up by me in all tests as a precaution. Flocculation was observed in a number of these controls at the same period (and usual in the same cases) when trouble was experienced in this direction with the Sachs-Georgi test. It disappeared, coincidently with the S.-G. serum control flocculations, on the adoption of a different glass-washing technique as already described.

As spontaneous flocculation occurs occasionally in certain sera, the routine use of a control tube would, however, appear to be indicated.

Flocculation of extract dilutions. — Some difficulty was at first experienced by heavy spontaneous flocculation of the extract after addition of distilled water. This, however, only occurred when larger quantities than 3 c.c. were diluted. When larger amounts were needed than the 25.5 c.c. (resulting from the dilution of 3 c.c.) this was accomplished by diluting further lots of 3 c.c. each. But the resulting dilutions were never really homogeneous

when viewed with a six-magnification lens, and it was found essential to put up extract controls with each batch of tests for purposes of comparison, though these are not indicated as necessary by the author of the test.

Use of three quantities of extract dilution. — When studying the test with the author in his laboratory at Ambrock, I observed that each serum was tested with one quantity of extract dilution only, namely, 0.8 c.c., although in a paper by the author which I read on my return to London the desirability is indicated of testing each serum with three quantities, namely, 0.5 and 0.8 and 1.5 c.c., the amount of undiluted serum in each tube being the same (0.2 c.c.). I wrote to Dr. Meinicke on the matter, and in his reply he advised me to use the three quantities and pointed out that, although rare, it yet sometimes occurred that a serum would react positively with 0.5 or 1.5 c.c. and fail to do so with 0.8 c.c. He further pointed out that sera containing hæmoglobin tended to react with 1.5 c.c. antigen dilution only. I accordingly adopted the three quantities as a routine, and with the exception of the first 60 tests all have been thus carried out.

I am able to confirm the author's statement that if one quantity of antigen dilution only is used, a positive reaction may occasionally be missed, but I did not find that any sera containing hæmoglobin reacted with the largest quantity of antigen dilution only.

Conclusion. — The technique of the Meinicke No. 3 test is simple to carry out and presents no difficulties other than those referred to above.

As carried out by the author of the method (with one tube only and without any controls) it is, of course, even simpler than with the various precautionary measures indicated above.

With the exception of a hand-lens, no special apparatus is required.

Wassermann test. — This was carried out by Method I, "Reports of the Special Committee upon the Standardisation of Pathological Methods" (Medical Research Committee).

In addition to the technique therein described, it was considered desirable to use a standardised suspension of sheep cells on each test-day and to read the results by means of a hæmoglobin scale.

Note. — In the preparation and standardisation of extracts for the S.-G., M. and Sigma reactions, considerable time and labour are involved. In regard to the antigen used in the W. R., on the other hand, as carried out in this investigation, these processes are comparatively simple.

II. Relative Consumption of Time

The time occupied (not including inactivation or incubation) in performing 23 tests by each method was observed as accurately as possible.

Sigma test.

Preparing tests for incubation	1 hour	56 min.
Reading at 20 hours and calculation of units in 7 positive cases.	35	—
Reading at 40 hours and calculation of units	7	—
Total.	2 hours	38 min.

S.-G. test

Preparing tests for incubation	35	min.
Reading	7	—
Total.	42	min.

M. No. 3 test.

Preparing tests for incubation	38	min.
Reading	12	—
Total.	50	min.

W. R.

Preparing tests for incubation, preparation of sensitized cell suspension, titration of complement and reading. Including standardisation of r. b. c. suspension and reading with hæmoglobin scale. Total	2 hours	47	min.
Excluding standardisation of r. b. c. suspension and hæmoglobin scale. Total	1 hour	30	min.

Total time occupied on 23 tests (including incubation as follows : Sigma 40-44 hours; S.-G. 18 hours; M. 18 hours; W. R. 1 1/2 hours) :

Sigma	42	hours	38	minutes	to 46 hours 38 minutes.
S.-G.	18	—	42	—	
M.	18	—	50	—	
W. R.	4	—	17	—	(with standardisation of r. b. c. and hæmoglobin scale)
and	3	—			(without standardisation of r. b. c. or hæmoglobin scale).

Approximate total time consumed by attendant on 23 tests :

Sigma	3	hours
S.-G.	1 1/2	—
M.	1 1/2	—
W. R.	4 1/2	—

In comparing the time expended in performing the Sigma test and the W. R. respectively, it is to be observed that, whilst this is approximately the same for a small number of tests, the Sigma test would occupy a relatively much longer time for a greater number. Thus, for, say, 70 sera the distribution of serum alone (9 tubes each) for the Sigma test would occupy 2 1/4 hours (23 sera occupied 45 minutes), whilst the time occupied in the W. R. for a similar number would be only slightly increased above that occupied for 23 tests.

When the first 5 tubes only of the Sigma test are employed (as has been suggested) the time occupied is approximately halved. But out of 449 tests, 13.5 % failed to give the end-point with 5 tubes only, and additional time would be occupied in the further testing of these specimens.

Again, whilst the preparation of 3 lots of Beta dilution and one lot of Alpha dilution of extract for 23 tests (9 tubes each) occupied 30 minutes, the preparation of the requisite amounts of extract dilutions for 70 tests would itself occupy 1 1/2 hours.

Taking one hundred tests as a basis, the complete W. R. could be performed on these in 3 to 4 hours, and with r. b. c. standardisation and hæmoglobin scale certainly within 5 hours. The mere preparation for incubation of a similar number of Sigma tests (9 tubes each) would occupy about 9 hours. If to this time is added about 2 hours for reading, the total period during which the serologist would be occupied actively on 100 Sigma tests would be 11 hours, as contrasted with a maximum of five hours for the same number of Wassermann tests, three hours for Sachs-Georgi tests, and 3 1/2 hours for Meinicke tests. These calculations do not, of course, include time spent in incubation.

III. Expense

Approximate initial cost of special apparatus for 100 tests (excluding apparatus common to all four methods) :

	£	s.	d.
Sigma	58	17	9
W. R.	25	6	0
S.-G.	16	15	0
M.	17	15	4

Approximate cost of reagents per 100 tests (excluding antigens and reagents—*e.g.*, saline common to all) :

Sigma approximately 2/5 (acetone 5d.; gas for distilled water, approximately 2/-).

W. R. 6/- (amboceptor 1/6; sheep's r. b. c. 1/-; guinea-pig 3/6).

S.-G. —

M. —

IV. Ease and Accuracy with which the Reaction can be Observed.

Sigma test. — Reference has already been made to the occurrence of non-specific flocculation, probably due to infection. It has occurred, when present, mostly in tubes 3 to 6 and, as previously stated, has usually been easily distinguished from true flocculation and has not greatly interfered with the readings, though the end-point has in some cases been obscured.

Until the observer has become thoroughly accustomed to the method, the reading of results is exceedingly fatiguing to the eye. It is only after considerable experience has been gained that the naked-eye readings down to S— or tr+ can be accurately gauged, and the finer degrees of flocculation are truly estimated with the hand-lens. Difficulty has been experienced on occasion in reading the results with densely cloudy sera, and the decision at 40-44 hours as to whether, in tube 1, the reading is t— or S+ may be one of no little nicety and importance, t— in treated cases being a positive reading.

Generally speaking, I should say that workers of like experience would obtain closely similar readings in any given case.

A disadvantage of this method is that, for reading the results, two persons are required—one for reading and the other for recording. Whilst such an arrangement is an advantage also in the other three tests, it is, in the Sigma test, practically essential.

S.-G. test. — The reading of this test by means of the agglutinoscope, as recommended by the authors, is exceedingly simple and accurate and free from strain.

It should also be mentioned that the instrument can be used with artificial light.

M. No. 3 test. — The reading of this test with a hand-lens and in the manner laid down by the author ("Man sieht von unten her durch das Röhrchen gegen die obere Wölbung eines Fensters, so dass die Lichtstrahlen schräg zur Blickrichtung in das Röhrchen einfallen") imposes a considerable strain on the observer. This is, of course, emphasized when three tubes and a serum control are used.

Slight degrees of flocculation are difficult to recognise on account of the fact that the antigen dilution is itself not clear. And although antigen control tubes were put up on each test-day, the comparison of these with doubtful tests was not found to be of much use in arriving at a decision.

In order, if possible, to abolish the strain of reading with the hand-lens, I inquired of Dr. Meinicke whether he had any objection to the use of the agglutinoscope provided it were fitted with a lens of the same power as the hand-lens ordinarily used. He replied as follows : "Persönlich ziehe ich die Betrachtung mit der Lupe dem Agglutinoskop vor. Sie können aber natürlich auch mit diesem Instrument ablesen, falls die Lupe schwach genug

ist. Etwas verstärkt werden die Resultate aber trotzdem erscheinen, da die Beleuchtung intensiver ist. In zweifelhaften Fällen können Sie ja auch jederzeit die einfache Lupenbetrachtung mit zu Rate ziehen. Wenn Sie das tun, dann ist dagegen die Benützung des Agglutinoskops wohl nichts einzuwenden".

I thought it desirable, however, in spite of the strain imposed, to continue my observations with the hand-lens only, chiefly in order to render my results strictly comparable to those of the other workers engaged upon this investigation.

W. R. — There can be no question that this is by far the easiest to read of the four reactions under review. And by means of the hæmoglobin scale a high degree of accuracy can be attained.

V. Percentage of Dubious Results.

Definition of dubious reading :

S.-G. : Up to and including +.
M ditto.
W. R. If inhibition of lysis of any degree in first tube, containing 3 MHD complement, together with complete lysis in second tube containing 5 MHD complement.
Sigma : 1.1 to 1.5 units at 40-44 hours.

The percentage of dubious readings in the last 449 tests was as follows :

S.-G.	=	5.3
M.	=	4.0
W. R.	=	5.3
Sigma	=	4.0

VI. The Extent to which the Method yields Quantitative Results.

Sigma test. — The essence of this method is the expression of the results in standard units.

As far as can be judged by comparison with the results obtained by the other methods, and without having access to the clinical records, these units connote quantitative results.

W. R., S.-G., and M. No. 3 tests. — These methods as ordinarily carried out yield only roughly quantitative results. By using falling quantities of serum, finer quantitative results, with a higher degree of accuracy, could doubtless be obtained, though this would, of course, add to the labour of the test.

In this investigation the use of a hæmoglobin scale for reading the results of the W. R. appeared to show that degrees of the reaction are brought to light thereby which would otherwise not have been obvious.

ANNEX

PASTEUR INSTITUTE, PARIS.

I. — Diphtheria Anti-Toxin

Ever since the first years of the introduction of serum treatment, the Ehrlich unit has been employed at the Pasteur Institute as standard for the measurement of the diphtheria anti-toxin.

The standard serum solution (glycerinated solution) was at first supplied to us by the Frankfort Institute; since the year 1913, however, the Public Health Service at Washington has been supplying us with its standard serum.

Researches carried out by us since 1913 have shown that these two standard sera tested by means of diphtheria toxins of various origin make it possible to determine L + under absolutely comparable conditions.

We give as an example the results of experiments carried out in March 1922.

Diphtheria Toxin J.

Washington Unit.		Frankfort Unit.
0.19	3 guinea pigs weighing from 250 to 260 grams survived.	3 guinea pigs weighing from 250 to 260 grams : 2 survived, 1 died of the infection.
0.20	3 guinea pigs weighing from 250 to 260 grams : 2 died in 4 days to 4 ½ days, 1 died in 6 ½ days.	3 guinea pigs weighing from 250 to 260 grams : 1 died in 3 ½ days, 1 died in 3 days, 1 died in 2 ½ days.
0.21	3 guinea pigs weighing from 250 to 260 grams : 1 died in 5 ½ days, 2 died in 2 ½ days.	3 guinea pigs weighing from 260 to 270 grams : 1 died in 4 ½ days, 1 died in 3 ½ days, 1 died in 2 ½ days.

II. — Tetanus Anti-Toxin

A. — *Comparison between the French and American Antitoxic Units.*

A comparative examination of various samples of anti-tetanic serum from the Pasteur Institute by means of the Pasteur test toxin and the Washington test toxin has shown that the *average* ratio between these two units was 1 to 53, taking the American standard serum as being 1 titre.

One serum, for instance, contains 4,500 French units to the cubic centimetre with the French test toxin; with the American test

toxin, 1,000th of a cubic centimetre (0.001) corresponds to the 10th of the unit, that is to say, 100 American units to the cubic centimetre. Ratio 1 to 45.

Another serum measures, with the same French test toxin, 5,500 units; with the Washington test toxin, the 900th part of a cubic centimetre (0.0011) corresponds to a 10th of the American unit, that is to say, 90 American units per cubic centimetre. Ratio 1 to 61.

The table of equivalence between the French and American units might be drawn up as follows :

French units per centimetre	American units per centimetre
10,000	200
8,000	160
5,000	100
4,000	80
1,000	20

B. — *Comparison between the French and the German Units.*

Comparison between the German unit (dry serum of which one cubic centimetre of a 1/17th solution corresponds to 100th part of IE). and the Pasteur test toxin shows that about a 25th (0.04) of 100th IE corresponds to the L + dose of the French test toxin. The ratio between the German unit and the French unit would therefore be about 1 to 2,500 (1).

The comparison between the French and the German units might be established approximately as shown in the table below.

French units per cubic centimetre	German units per cubic centimetre
11,000 to 12,500	5 IE
8,000 to 10,000	4 IE
4,500 to 5,000	2 IE
2,000	1 IE
1,000	0.4 to 0.5 IE

If we compare the tables for the French unit with those for the American and German units, taking the American standard serum as being 1 titre, it will be seen that the *approximate ratio* between

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(1) Dilutions at 1/10, 1/15, 1/20, 1/40, 1/50, 1/100, 1/200 of the test dose of the German serum (1/100 IE) are mixed with the standard dose of French toxin (toxin B O milligram.07) and injected subcutaneously after an hour's contact into mice weighing 15 grams :

1/10 to 1/20 no tetanus.
1/40 death after 2 ½ days.
1/50 death in 24 hours.
1/100 to 1/200 death in less than 24 hours.

The same experiment was made with dilutions of 1/18, 1/20, 1/25, 1/30, 1/40 of the test dose of the German serum. The results were as follows :

1/18 no tetanus; 1/20 local tetanus; 1/25 death in 2 ½ days.
1/30 death in 1 ½ and 2 days 3 ½ —
1/35 to 1/40 death in 1 ½ days. 4 ½ —

the three units are as 1 to 53 in the case of the American and French units, 1 to 50 in the case of the American and German units and 1 to 2,500 in the case of the German and French units.

French units to the cubic centimetre	American units to the cubic centimetre	German units to the cubic centimetre
11,000 to 12,500		5
10,000	200	4
8,000	160	
5,000	100	2
4,000	80	
2,000	40	1
1,000	20	0.4 à 0.5

These comparative tests should have been repeated and more accurate results obtained, but we were not able to do so since the quantities of standard serum supplied to us were not sufficient.

The differences in the results obtained by different persons in carrying out the comparative tests with the American, German and French units are, no doubt, in the main, due to the variety of methods employed.

In order to obtain parallel values, it would be desirable to adopt a uniform method of carrying out the tests.

PRINTED BY BERGER-LEVRAULT, NANCY-PARIS-STRASBURG

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1922

International Conference on the Standardisation of Sera and Serological Tests, 2d, Paris, 1922
Reports on serological investigations

Biological & Medical

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